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2 **Multicenter Evaluation of the QIAstat-Dx Respiratory Panel for the Detection of Viruses**
3 **and Bacteria in Nasopharyngeal Swab Specimens**

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18 Running title: Multicenter Evaluation of the QIAstat-Dx Respiratory Panel

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20 **ABSTRACT** The QIAstat-Dx Respiratory Panel (QIAstat-Dx RP) is a multiplex *in vitro*
21 diagnostic test for the qualitative detection of 20 pathogens directly from nasopharyngeal swab
22 (NPS) specimens. The assay is a performed using a simple sample to answer platform with
23 results available in approximately 69 minutes. The pathogens identified are adenovirus,
24 coronavirus 229E, coronavirus HKU1, coronavirus NL63, coronavirus OC43, human
25 metapneumovirus A+B, influenza A, influenza A H1, influenza A H3, influenza A H1N1/2009,
26 influenza B, parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3, parainfluenza
27 virus 4, rhinovirus/enterovirus, respiratory syncytial virus A+B, *Bordetella pertussis*,
28 *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*. This multicenter evaluation provides
29 data obtained from 1994 prospectively collected and 310 retrospectively collected (archived)
30 NPS specimens with performance compared to the BioFire FilmArray Respiratory Panel version
31 1.7. The overall percent agreement between QIAstat-Dx RP and the comparator testing was
32 99.5%. In the prospective cohort, the QIAstat-Dx RP demonstrated a positive percent agreement
33 of 94.0% or greater for detection of all but four analytes: coronaviruses 229E, NL63 and OC43,
34 and rhinovirus/enterovirus. The test also demonstrated a negative percent agreement of $\geq 97.9\%$
35 for all analytes. The QIAstat-Dx RP is a robust and accurate assay for rapid, comprehensive
36 testing for respiratory pathogens.
37

38

39 **INTRODUCTION**

40 Respiratory infections are common and contribute significantly to morbidity and
41 mortality. They are also costly, being one of the leading reasons for healthcare visits (1, 2).
42 Because infections with respiratory pathogens often result in symptoms that overlap between
43 many causative agents, a definitive diagnosis requires laboratory testing. Therefore the approach
44 of syndromic testing has been widely adopted with testing for multiple agents of upper
45 respiratory infection at the same time with a single test. This panel-based approach can simplify
46 ordering and laboratory workflow while improving sensitivity and time to result compared to
47 older, conventional testing methods.

48 From a clinical perspective, use of syndromic diagnostics can facilitate better
49 antimicrobial stewardship by allowing antimicrobial or antiviral therapy to be given in a timely
50 and appropriate manner (3, 4). The misuse of antibiotics in cases of viral respiratory infections is
51 a common problem and a rapid result detecting a viral pathogen may prevent the unnecessary use
52 of antibiotics. Rapid diagnosis of respiratory infections can also shorten times in the emergency
53 room, decreased length of stay or prevent hospitalization and allow improved patient cohorting
54 to prevent nosocomial infections (3, 5-9).

55 The first multiplex respiratory panel was cleared by the FDA in 2009. This has been
56 followed by a number of such syndromic assays. These panels vary in the number of analytes
57 detected and the time to result but most are designed to be simple to use and require little hands
58 on time (10). All current commercial multiplex assays of 5 or greater analytes include viral
59 pathogens such as influenza A and B, respiratory syncytial virus, human metapneumovirus,
60 adenovirus, parainfluenza virus, and rhinovirus/enterovirus. A smaller number of these panels

61 include bacterial pathogens such as *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and
62 *Bordetella* species (10).

63 In this study, data are presented for a multicenter clinical evaluation of a new multiplex
64 respiratory panel, the QIAstat-Dx® Respiratory Panel (QIAstat-Dx RP). The QIAstat-Dx RP is
65 a multiplexed real-time PCR test intended for use with QIAstat-Dx system for the simultaneous
66 qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in
67 nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections.
68 Each QIAstat-DX RP cartridge is run on an analyzer which consists of at least one analytical
69 module for individual cartridge loading and one operational module with touch screen and
70 integrated software. Up to 4 analytical modules can be connected with one operational module
71 (Figure 1). The following pathogens types and subtypes are identified: adenovirus; coronaviruses
72 229E, HKU1, NL63, OC43; human metapneumovirus A+B; influenza A; influenza A H1;
73 influenza A H3; influenza A H1N1/2009; influenza B; parainfluenza viruses 1, 2, 3, and 4;
74 rhinovirus/enterovirus; respiratory syncytial virus A+B; *Bordetella pertussis*; *Chlamydia*
75 *pneumoniae*; and *Mycoplasma pneumoniae*. Testing was performed on residual NPS collected in
76 transport media. Both a prospective and retrospective arm of the study are included. For all 20
77 analytes, performance calculations are based on comparison to an FDA-cleared/approved test.

78

79 MATERIALS AND METHODS

80 **Prospective Clinical Specimens.** The study was conducted at six geographically distinct
81 sites in the U.S. and Europe (Nationwide Children's Hospital – Columbus, OH, Hennepin
82 County Medical Center – Minneapolis, MN, Indiana University School of Medicine –
83 Indianapolis IN, Laboratory Alliance of Central New York – Liverpool, NY, TriCore Reference

84 Laboratories – Albuquerque, NM, and University of Copenhagen - Hvidovre, Denmark).

85 Specimens were prospectively enrolled over a period of approximately 17 months (December

86 2017-April 2019) and tested either fresh or after being frozen at $\leq -70^{\circ}\text{C}$. Specimens meeting the

87 following inclusion criteria were selected: specimen was an NPS collected in transport media for

88 standard of care (SOC) testing. The transport media used in this study were as follows:

89 Universal Transport Medium, Copan Diagnostics, Brescia, Italy and CA, USA; MicroTest M4,

90 M4RT, M5, M6, Thermo Fisher Scientific, MA, USA; BD Universal Viral Transport, Becton

91 Dickinson, NJ, USA; Universal Transport Medium, HealthLink Inc., FL, USA; Universal

92 Transport Medium, Diagnostic Hybrids, OH, USA; V-C-M Medium, Quest Diagnostics, NJ,

93 USA; UniTranz-RT Universal Transport Media, Puritan Diagnostics, ME, USA. The specimen

94 had to have adequate residual volume ($\geq 2.0\text{mL}$ for U.S. sites and $\geq 1.5\text{mL}$ for Hvidovre

95 Hospital), and had been held at room temperature for less than or equal to 4 hours, at 4°C for less

96 than or equal to three days, or at -20°C or -70°C frozen for more than three days before

97 enrollment. A waiver of informed consent requirement was obtained from the Institutional

98 Review Boards (IRBs) at each study site for the use of residual deidentified NPS specimens.

99 **Retrospective (Archived) Clinical Specimens.** Preselected frozen archive specimens

100 were enrolled based on identification of specific positive targets using SOC testing at each study

101 site. Specimens were thawed and tested at each study site, in blinded fashion, with both the

102 QIAstat-Dx RP and the comparator assay, BioFire FilmArray Respiratory Panel version 1.7

103 (FARPV1.7). If the comparator assay did not confirm the preselected target as positive, the

104 specimen was excluded from the data analysis for that target.

105 **Clinical and demographic data.** Data were collected for both prospective and
106 retrospective specimen; the information included hospitalization status at the time of specimen
107 collection, date of specimen collection, subject sex, and subject age at time of collection.

108 **QIAstat-Dx Respiratory Panel.** The panel includes testing for detection of adenovirus,
109 coronavirus 229E (CoV 229E), CoV HKU1, CoVNL63, CoV OC43, human metapneumovirus
110 A+B (hMPV), influenza A (FLU A), FLU A H1, FLU A H3, FLU A H1N1/2009, influenza B
111 (FLU B), parainfluenza virus 1 (PIV 1), PIV 2, PIV 3, PIV 4, human rhinovirus/enterovirus
112 (RV/EV), respiratory syncytial Virus A+B (RSV), *Bordetella pertussis*, *Chlamydomphila*
113 *pneumoniae* and *Mycoplasma pneumoniae*. Approximately 300 µl of specimen was tested
114 according to manufacturer's instructions (11). The QIAstat-Dx Respiratory Panel Cartridge and
115 platform consists of automated nucleic acid extraction, reverse transcription, polymerase chain
116 reaction (PCR), and fluorescence detection with results analysis in approximately 69 minutes per
117 run (i.e., per specimen); Figure 1 shows the instrument workflow. The PCR is run to 40 cycles
118 and the fluorescence readings are analyzed by the Result Calling Algorithm (RCA) to determine
119 positive or negative calls. The cartridge includes a full process Internal Control which is titered
120 MS2 bacteriophage in dried form that is rehydrated upon specimen loading. This control material
121 verifies all steps of the analysis process.

122 The QIAstat-Dx RP Analyzer performs automated result analysis with each target in a
123 valid run reported as positive or negative. The qualitative results are displayed on the instrument
124 screen and can be printed. If the internal control fails, the software automatically will provide a
125 result for targets that test positive but the other panel targets will result as 'Invalid'. Within the
126 software is a report to display the amplification curve for each target, for which the cycle
127 threshold (Ct) and endpoint fluorescence value are provided on the final printed report. This

128 study was conducted with an Investigational Use Only (IUO) version of the QIAstat-Dx RP that
129 is identical to the final FDA-cleared/CE-IVD marked version.

130 **Comparator Testing.** Comparator testing consisted of FilmArray Respiratory Panel
131 version 1.7 (FARPV1.7) testing for all targets, with testing performed at the source laboratory.
132 The assay detects adenovirus, coronavirus 229E, coronavirus HKU1, coronavirus NL63,
133 coronavirus OC43, human metapneumovirus, influenza A, influenza A H1, influenza A H3,
134 influenza A H1-2009, influenza B, parainfluenza virus 1, parainfluenza virus 2, parainfluenza
135 virus 3, parainfluenza virus 4, rhinovirus/enterovirus, respiratory syncytial virus, *Bordetella*
136 *pertussis*, *Chlamydomphila pneumoniae* and *Mycoplasma pneumoniae*.

137 **Results and Discrepant Analysis.** A QIAstat-Dx Respiratory Panel result was considered a true
138 positive (TP) or true negative (TN) only when it agreed with the result from the comparator
139 method (FARPV1.7). Discrepant analysis ensued when results were discordant, i.e. false positive
140 (FP) or false negative (FN) results.

141 Discrepant analysis for all panel targets excluding *Bordetella pertussis* was performed
142 using the NxTAG® Respiratory Pathogen Panel on the Luminex® MAGPIX® Instrument, at
143 one clinical study site (Indiana University School of Medicine). For *B. pertussis* discordant
144 analysis, the VERIGENE® Respiratory Pathogens Flex Test (RP Flex) was used to detect and
145 differentiate the following *Bordetella* species, *Bordetella parapertussis/bronchiseptica*,
146 *Bordetella holmesii*, and *Bordetella pertussis*. This testing was performed at one clinical study
147 site (Laboratory Alliances of Central New York).

148 Note that the performance data for sensitivity/positive percent agreement (PPA) and
149 specificity/negative percent agreement (NPA) presented in this manuscript consist of unresolved

150 data as presented in the package insert for the FDA-cleared test; discrepancy investigation is
151 provided but was not used to recalculate performance data.

152 **Workflow and Time to Results:**

153 For workflow analysis, the operating procedures were compared to determine differences
154 in the number of steps required for set up (11, 12). A total of 20 specimens were set up for
155 analysis by both methods and the time required from beginning set up to loading into the
156 instrument was timed. In addition, a random sampling of 50 results generated on the same
157 specimen run on the QIAstat-Dx RP and the FARPv1.7 was analyzed and the average time to
158 result for each platform was calculated.

159 **Statistical Analysis.** Binomial two-sided 95% confidence intervals were calculated
160 using the Wilson Score Method. Differences in median Ct values were determined using Mood's
161 median test.

162

163 **RESULTS**

164 **Demographics.** A total of 2,304 specimens (1994 prospective and 310 retrospective)
165 were included in data analysis for both arms of the study collected from a range of
166 geographic/demographic populations (Table 1). Overall, specimens included slightly more
167 female than male subjects (53%, 1,222/2,304 and 47%, 1,082/2,304, respectively). The
168 specimens were from various age groups: 33% of the specimens were from children aged 5 and
169 under, 14% were from those aged 6-21, 17% were from adults aged 22-49, and 36% were from
170 adults over the age of 50. For treatment setting, 32.7%, (754/2,304) were obtained from
171 hospitalized patients, 3.3% (75/2304) from those visiting the emergency department, 7.0%
172 (161/2,304) were admitted to the ICU and 43.9 % (1,012/2,304) were obtained from subjects

173 seen in an outpatient setting. For 302 (13.1%) specimens, the location was other than those listed
174 or unknown.

175 **QIAstat-Dx RP test performance.** There were a total of 2,342 specimens originally
176 enrolled for both arms of the study (prospective – 2018 [1,111 frozen, 907 fresh; retrospective] –
177 324 archived frozen). A total of 24 prospective specimens were excluded for reasons related to
178 sample stability or test performance. Fourteen retrospective samples were withdrawn because the
179 target of interest did not confirm on repeat testing with the comparator assay (FARV1.7).

180 Of the 1,994 prospective specimens tested and analyzed during the clinical evaluation,
181 95.9% (1,912/1,994) yielded valid results on the first attempt (i.e., first loaded cartridge). Invalid
182 or no result were obtained for the remaining 82 specimens (4.11%). Forty-two (42) specimens
183 were invalid due to cartridge internal control failure (2.11%). Of these, 20 (1.00%) provided a
184 result for positively detected targets and 22 (1.10%) had no detections. For 40 (2.00%)
185 specimens no results were obtained due to incomplete runs. Of these, one specimen was aborted
186 by the user (0.05%), 21 were due to instrument errors (1.05%) and 18 were due to cartridge
187 related errors (0.90%).

188 Seventy-two (72) of the 82 initially failed (no results or invalid) specimens yielded valid results
189 after a single retesting using a new cartridge/specimen. The remaining 10 specimens failed on
190 the second attempt: two due to cartridge failures, one due to instrument errors and seven due to
191 internal control failures. Of these internal control failures, detected pathogens were reported for
192 four specimens. Thus six samples (6 of 1994 = 0.3%) did not provide valid results after a single
193 retest, yielding a 99.7% success rate after a single retest.

194 **Summary of QIAstat-Dx RP findings.**

195 **Prospective Specimens.** The QIAstat-Dx RP detected at least one analyte in 1166 of
196 1,994 specimens tested, yielding an overall positivity rate of 58.5% (Table 1) The highest
197 detection rate was seen in young children (≤ 5 years of age; 24.1%) followed by those >50 years
198 of age (19.5%).

199 The summary of prospective performance characteristics for individual QIAstat-Dx RP
200 targets is presented in Table 2. PPA and NPA were calculated with respect to the comparator
201 method along with 95% CI. The QIAstat-Dx RP demonstrated a PPA of 91.2% or greater for all
202 but three analytes. For FLU A H1, no PPA could be calculated. The three analytes demonstrating
203 a PPA $< 91.2\%$: - all were CoV: CoV 229E (88.9%), CoV NL63 (85.1%), and CoV 43 (89.7%).
204 Additionally, nine analytes demonstrated a lower bound of the two-sided 95% CI $< 80.0\%$ due to
205 few or no observations in the study. Overall, the QIAstat-Dx RP demonstrated a NPA of \geq
206 97.9% for all analytes, with lower bounds of the two-sided 95% CI of $\geq 97.1\%$.

207 The QIAstat-Dx RP detected a total of 191 specimens with distinctive multiple organism
208 detections representing 9.6% of all prospective samples. There were 166 double infections, 22
209 triple infection and 3 quadruple infections. The rate of multiple detection by age groups was:
210 79.1% (151/191) for <6 years; 6.3% (12/191) for 6-21 years; 7.3% (14/191) for 22-49 years; and
211 7.3% (14/191) for >49 years. The three pathogens most prevalent in the multiple detections were
212 RV/EV (108/191, 56.5%), RSV (77/191, 40.8%) and adenovirus (53/191, 27.7%).

213 **Retrospective Specimens.** Performance characteristics for the retrospective specimens
214 are presented in Table 3. The QIAstat-Dx RP detected at least one analyte in 299 of 310
215 specimens tested, yielding an overall positivity rate of 96.5% (Table 1). For the 11 negative
216 samples, comparator testing was positive for the pathogen of interest, with retesting accruing on
217 same freeze-thaw cycle as the testing with QIAstat-Dx RP. With this smaller archived sample

218 set, PPA was >90% for all but 4 targets. Also, the lower bounds of the 95% CI for the PPA were
219 lower than the prospective group due to fewer samples tested. Values for NPA were all above
220 95% for all 20 targets. As these samples were pre-selected, prevalence was not evaluated.

221 **Comparator Analysis and Discrepancy Investigation.** There were a total of 45,895
222 analyzable QIAstat-Dx RP pathogen results for the 2,304 specimens (prospective and
223 retrospective). The overall percent agreement between QIAstat-Dx RP and the comparator
224 testing was 99.5% (45,662/45,895). There were 2,075 detected pathogen results with the
225 QIAstat-Dx RP; the comparator method was positive for 2,026 pathogen detections. The overall
226 PPA with respect to the comparator method was 95.5% (1,934/2,026). Data for the median Ct
227 values for all positive detections for the QIAstat-Dx RP are presented in the supplemental
228 material (Table S1).

229 There were 43,871- “not detected” results with the QIAstat-Dx RP; the comparator
230 method was negative for 43,920 analytes. The overall NPA with respect to the comparator
231 method was 99.7% (43,728/43,869).

232 For the viral analytes, QIAstat-Dx RP detected a total of 1,923 viral analytes compared to
233 1,880 for FARPv1.7. Using the comparator as truth, the overall PPA and NPA were 95.5%
234 (1,795/1,880) and 99.7% (32,101/32,117) respectively. Using the comparator as truth, the overall
235 PPA and NPA were 92.3% (36/39) and 99.9% (6402/6409) respectively for all bacterial targets.

236 Using comparator testing as the true result, there were 141 FP detections and 92 FN
237 detections overall; additional discrepancy analysis was performed for 214 (91.8%) of these false
238 detections. For 62 of the 141 FP cases (44%), along with 30 of the 92 FN cases (33%), there was
239 supportive evidence for the QIAstat-Dx RP result, bringing the adjudicated overall concordance

240 for the positive and negative results to 98.5% and 99.7%, respectively. A summary of the
241 discrepancy investigation is presented in Table 4.

242 **Workflow and time-to-results.** A review of the procedure showed that the steps for the
243 set up the pouches up to loading in the instrument did differ between the two platforms with the
244 specimen being pipetted directly into the QIAstat-Dx while the FA Rpv1.7 required addition of
245 both sample and a diluent using injection vials for reagent hydration and sample preparation in
246 addition to a transfer pipette for manipulating the specimen. Timed studies for set up of 20
247 pouches by two operators from specimen to loading took on average 35 seconds for the QIAstat-
248 Dx RP and 115 seconds for the FARpv1.7.

249 The average time to results for the 50 paired runs was as determined by each instrument was
250 69.1 ± 0.8 mins for QIAstat-Dx RP and 63.4 ± 0.5 mins for FARpv1.7.

251 DISCUSSION

252 This evaluation of the QIAstat-Dx RP demonstrated the performance of the test in a large
253 multicenter study using 2,304 residual NPS specimens with 45,895 results generated. This new
254 respiratory multiplex panel can be used to aid in diagnostic testing of respiratory infections. In
255 this trial, the number of prospective positive detections was relatively high for most pathogens
256 with the exception of CoV 229E, PIV 4, and *B. pertussis* (all with $N < 5$). No detections were
257 found for *C. pneumoniae*, PIV 1 and FLU A H1, which was not circulating during the study
258 period. The QIAstat-Dx RP testing system was shown to be reliable with few failures (95.3%
259 success on the initial test attempt for the prospective samples tested) and rapid with results
260 available in approximately 69 minutes. The data presented here along with testing of contrived
261 specimens were used as part of the regulatory submissions for the QIAstat-Dx RP which
262 received *de novo* 510 (k) clearance in the U.S. in May 2019 (11).

263 Taken in total, the QIAstat-Dx RP performance was comparable to the FARPv1.7 with an
264 overall percent agreement of 99.5%. In the prospective cohort, the QIAstat-Dx RP demonstrated
265 a PPA of 94.0% or greater for detection of all but four analytes: CoV 229E, NL63, and OC43,
266 and RV/EV. The test also demonstrated a NPA of $\geq 99.6\%$ for all analytes. The discordant
267 analysis showed that both assays appear to generate “false” results as would be expected. The
268 NxTAG assay used for discordant analysis is very similar to both these assays being a multiplex
269 respiratory panel. So for the discordant analysis, truth was determined by a best of two out of
270 three test results.

271 Viruses are a common cause of respiratory infections in both adult and pediatric
272 population which was also seen in our study cohort. While the QIAstat-Dx RP had a higher
273 number of positive viral detections overall compared to FARPv1.7 (1,645 versus 1,610), for
274 individual targets, there was increased sensitivity found with both assays depending on the
275 analyte. Viral detections were notably higher than those of the bacterial targets among the
276 prospective specimens (1645 viral vs. 39 bacterial detections).

277 Rhinovirus/Enterovirus. The most common viral analyte was RV/EV with a total of 304
278 positive detections. The extensive diversity within the rhinoviruses means that most molecular
279 assays, including QIAstat-Dx RP and the comparator assay, target the 5'UTR. This region is
280 highly conserved among all rhinoviruses and enteroviruses, causing cross-reactivity between
281 assays for the two viruses and making their differentiation difficult (13). RV/EV was also the
282 target showing the highest number of discordant results. The discordant specimens were
283 analyzed with the NxTAG® Respiratory Pathogen Panel, another FDA-cleared multiplex that
284 targets the 5'UTR. Therefore, no definitive resolution of the type of virus (rhinovirus versus
285 enterovirus) was made for the FP and FN samples.

286 Adenovirus. For adenoviruses, QIAstat-Dx RP is designed to detect genogroups B, C,
287 and E, the types most commonly associated with respiratory infections. It will also detect, to
288 some degree, genotypes A, D, F and G as evidenced by contrived testing with typed strains (11).
289 The FARPv1.7 was also designed for detection of genotypes B, C, and E. Both tests use the
290 hexon gene as the target. The differences in performance between these two tests in the present
291 study may related to specific primer and probe sequence differences or the level of sensitivity
292 and specificity of the assays for the many different serotypes of adenovirus (Prior studies have
293 demonstrated that a significant number of adenoviruses from upper respiratory samples may be
294 in genogroups A, D, and F and could be missed by tests that are not designed for broad coverage
295 of adenoviruses. (14, 15). Some recent data suggest that broadened inclusivity targeting the non-
296 respiratory types may improve clinical assay performance (16).

297 Coronavirus. The QIAstat-Dx RP assay has 4 distinct targets for detection of CoV. Three
298 of four of these targets (Table 2) had PPA <90% which were the lowest for all analytes in the
299 prospective analysis. In contrast, the retrospective CoV specimens showed better positive
300 agreement with all targets > 96% (Table 3). It is unclear why there were differences in
301 performance in the two arms of the study. There were a relatively low number of positive
302 detections in general. In addition, the FP sample did have significantly higher Ct values than the
303 TP for all samples (Supplemental Table 1), suggesting that the FP were related to low level of
304 virus. The level of virus in these FN specimens cannot be estimated as no semi-quantitative value,
305 such as Ct, is provided with the FARPv1.7.

306 Influenza viruses. Among the viruses detected in this multiplex panel, there is substantial
307 evidence that the rapid molecular diagnosis of influenza virus infections impacts patient
308 outcomes for both adult and pediatric populations (3, 6, 9). The QIAstat-Dx RP has a total of 4

309 targets for detection of FLU A: a pan-influenza A target and specific targets for 3 subtypes -
310 Influenza A H1, Influenza A H1 2009, and Influenza A H3. Of the 251 Influenza A positive
311 detections, a total of 248 (98.8%) had additional subtype specific detections (85 specific
312 detections for H1N1 2009 and 163 for H3). There were no detections of seasonal H1N1. Three
313 (1.2%) Influenza A positives had no associated specific detections. This could be due to virus
314 levels below the limit of detection for the type specific assays. However, it could indicate the
315 detection of a novel influenza A type and this should be considered when seen in clinical use.(17,
316 18) For FLU B virus there is a single target designed to detect the two sublineages of the virus
317 [B/Victoria/2/87–like (Victoria lineage) and B/Yamagata/16/88–like (Yamagata lineage)]. There
318 were 7 FN results with the QIAstat-Dx RP and 1 with FARPv1.7, which may reflect differences
319 in sensitivity related to the viral strains. Because the comparator does not provide any semi-
320 quantitative value it is difficult to determine the relative level of virus in the 7 QIAstat-Dx FN;
321 however, the 1 FN for the FARPv1.7 had a Ct value of 20.4, suggesting it did have a significant
322 amount of virus present.

323 A relatively low number of bacterial detections were found in the prospective cohort as
324 has been seen in other studies with multiplex testing (16, 19). The most common of the
325 bacterial targets was *M. pneumoniae* with 24 detections, more than the 19 detected with
326 FARPv1.7. However, it should be noted that use of an NP specimen for detection of *M.*
327 *pneumoniae* may be suboptimal particularly when diagnosing lower respiratory tract infection
328 (20, 21). For *B. pertussis*, discrepancy between the QIAstat-Dx RP and the comparator method
329 were not unexpected as the QIAstat-Dx RP targets the multi-copy insertion sequence (IS481)
330 which is present in several *Bordetella* species (*B. pertussis*, *B. holmesii*, and *B. bronchiseptica*)
331 whereas the comparator targets the single-copy promoter region of the pertussis toxin gene and is

332 designed to be specific to the detection of *B. pertussis*. Use of the single-copy toxin gene target
333 has been shown to be less sensitive than IS481 (22, 23). The assay used for discordant analysis
334 calls out the individual *Bordetella* species (*B. pertussis*, *B. parapertussis* and *B. holmesii*). The *B.*
335 *pertussis* target is also the toxin promoter region so would be a single-copy gene. There were a
336 total of 6 QIAstat-Dx RP FP results for *B. pertussis* for both arms of the study (Table 4). Five of
337 these samples were available for further analysis; only one (1) confirmed using the discordant
338 testing with at Ct value of 31.6. In examining the Ct values for the all detections in the clinical
339 trial, the discordant detections had a significantly higher median Ct value than the concordant
340 positive detections (TP median Ct =26.2 vs. FP median Ct =33.0, p value=0.008, TABLE S1).
341 Thus some of the FP may have been missed by both the comparator and discordant analysis
342 assays based on the lower sensitivity of a single copy gene target.

343 As with other multiplex respiratory panels, the QIAstat-Dx RP allows for detection of
344 multiple pathogens representing co-infections. The rate of co-detections was highest in the
345 pediatric patients <6 years of age and the most common analytes in the codetections were
346 RV/EV, RSV and adenovirus. Similar findings have been reported for other multiplex respiratory
347 panels (16, 19). More data is needed on the impact of codetections on outcomes however it may
348 be useful for infection control purposes to allow better cohorting or isolation of infected patients
349 (24).

350 The QIAstat-Dx RP workflow is very simple and the footprint of the instruments is small
351 measuring 20.3cm(width) x 32.6cm (height) x 51.7cm (depth) for 1 Operational Module plus 1
352 Analytical module (8.0in w x 12.8in h x 20.4in d; see Figure 1). In comparison to FARPv1.7,
353 QIAstat-Dx RP involves only one step to pipette the specimen into the cartridge for loading as
354 there is no buffer added nor any other manipulations required. This lessens manipulation and

355 may help to reduce contamination. The run times are similar lasting on average 69 and 63
356 minutes for the QIAstat-Dx RP and FARPv1.7 respectively. Both platforms allow for testing of
357 one pouch at a time per module. In terms of reliability, the initial rate of invalid or no results on
358 first testing for the prospective samples was 4.1% and after a second test 0.7% This invalid rate
359 is comparable to other multiplex platforms currently available (16). A significant benefit of the
360 system allows the user to obtain a Ct value for each detected pathogen and the internal control.
361 The comparator assay does not allow the user to see Ct values. These values, while not truly
362 quantitative, do allow a semi-quantitative assessment of target amount that can be useful in
363 trouble-shooting or other quality control measures.

364 There are some limitations with this study. For the prospective arm, some specimens
365 were tested fresh but some were frozen at $\leq -70^{\circ}\text{C}$ prior to testing. However, data indicated that
366 the frozen storage did not significantly affect performance (11). The study period bridges 17
367 months and includes two respiratory seasons; however variations in circulating strains,
368 particularly influenza A viruses, may be limited. Because the prevalence of some analytes was
369 low in the prospective cohort, frozen retrospective samples were used to increase the numbers
370 for positive detections. As stated above, freeze thawing did not appear to affect performance in
371 terms of prevalence. However for the retrospective samples, all were tested with both the test and
372 comparator assays on the same freeze-thaw cycle to remove this as a confounder. Overall the
373 percentage of discrepant results versus the comparator methods was low.

374 In summary, the QIAstat-Dx RP demonstrated good comparative performance in this
375 large multicenter clinical trial and represents a new alternative for multiplex respiratory testing.
376 It is a robust and accurate assay for rapid and comprehensive testing for respiratory pathogens
377 from nasopharyngeal swab specimens.

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465

466 **FIGURE 1** QIAstat-Dx Respiratory Panel Assay Workflow

467 **TABLE 1** Demographics and Positivity Rate for QIAstat-Dx Respiratory Panel: For all
 468 Prospective and Retrospective Samples and By Age Groupings

	Prospective Samples (n=1994)		Retrospective Samples (n=310) ^a	
Demographics and Location				
	No.	% of Total	No.	% of Total
Male	924	46.3	158	50.8
Female	1070	53.7	152	49.2
Outpatients	788	39.5	224	72.3
Hospitalized	686	34.4	68	21.9
Emergency	67	3.4	8	2.6
ICU	153	7.7	8	2.6
Other/Unknown	300	15.0	2	0.6
Overall Positivity and Co-detections				
	No.	% of Total	No.	% of Total
Negative Samples	828	41.5	11	3.5
Positive Samples	1166	58.5	299	96.5
Single Detections	800	40.1	222	71.6
Co-Detections	366	18.4	77	24.8
Positivity by Age Grouping				
	No.	% of Total	No.	% of Total
≤5 years (n=627)	481	24.1	137	44.2
6-21 years (n=239)	123	6.2	80	25.8
22-49 years (n=330)	174	8.7	48	15.5
50+ years (n=798)	388	19.5	34	11.5

469 ^aAll retrospective samples were chosen from frozen archives based on initial standard of care
 470 testing and retested with the QIAstat-Dx RP and comparator.

471
 472

473 **TABLE 2** Performance Summary of the QIAstat-Dx Respiratory Panel for Prospective

474 Specimens^a

Analyte	N ^b	Positive Percent Agreement			Negative Percent Agreement		
		TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Viruses							
Adenovirus	1986	86/90	95.6	89.1-98.3	1880/1896	99.2	98.6-99.5
Coronavirus 229E	1984	8/9	88.9	56.5-98.0	1975/1975	100	99.8-100.0
Coronavirus HKU1	1984	51/52	98.1	89.9-99.7	1925/1932	99.6	99.3-99.8
Coronavirus NL63	1985	40/47	85.1	72.3-92.6	1936/1938	99.9	99.6-100.0
Coronavirus OC43	1984	26/29	89.7	73.6-96.4	1951/1955	99.8	99.5-99.9
Human Metapneumovirus	1985	115/122	94.3	88.6-97.2	1858/1863	99.7	99.4-99.9
Rhinovirus/Enterovirus	1986	268/294	91.2	87.4-93.9	1656/1692	97.9	97.1-98.5
Influenza A	1978	242/244	99.2	97.0-99.8	1725/1734	99.5	99.0-99.7
Influenza A H1	1984	0/1	0.0	0.0-79.3	1983/1983	100.0	99.8-100.0
Influenza A H1N1\2009	1983	80/81	98.8	98.3-99.8	1897/1902	99.7	99.4-99.9
Influenza A H3	1981	156/157	99.4	93.3-99.8	1817/1824	99.6	99.2-99.8
Influenza B	1983	122/129	94.6	89.2-97.3	1853/1854	99.9	99.7-100.0
Parainfluenza Virus 1	1984	16/17	94.1	73.0-99.0	1964/1967	99.8	99.6-99.9
Parainfluenza Virus 2	1984	2/2	100.0	34.2-100.0	1982/1982	100.0	99.8-100.0
Parainfluenza Virus 3	1987	111/113	98.2	93.8-99.5	1869-1874	99.7	99.4-99.9
Parainfluenza Virus 4	1984	3/3	100.0	43.8-100.0	1979-1981	99.9	99.6-100.0
Respiratory Syncytial Virus	1985	212/220	96.4	93.0-98.1	1760/1765	99.7	99.3-99.9
Bacteria							
<i>Bordetella pertussis</i>	1984	3/3	100.0	43.8-100.0	1975/1981	99.7	99.3-99.9
<i>Chlamydia pneumoniae</i>	1984	5/5	100.0	56.6-100.0	1978/1979	99.9	99.7-100.0
<i>Mycoplasma pneumoniae</i>	1984	19/19	100.0	83.2-100.0	1960/1965	99.7	99.4-99.9

475 ^aThese data are presented based on comparator assay (BioFire FilmArray Respiratory Panel
476 version 1.7) only and do not reflect any discordant analysis. Both the fresh and frozen samples
477 are presented together as no differences in performance were determined statistically (data not
478 shown).

479 ^bIn instances where the internal control failed and was not resolved upon repeat, any target that
480 was "Detected" was maintained within the data set and used in performance calculations. All
481 targets that were not detected were considered failed and excluded from the data analysis
482 therefore the final "N" will vary by analyte.

483

484 **TABLE 3** Performance Summary of the QIAstat-Dx Respiratory Panel for Retrospective

485 Specimens^a

Analyte	No, ^b	Positive Percent Agreement			Negative Percent Agreement		
		TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Viruses							
Adenovirus	313	9/9	100.0	70.1-100.0	297/304	97.8	95.4-98.9
Coronavirus 229E	313	26/27	96.3	81.7-99.3	286/286	100.0	98.7-100.0
Coronavirus HKU1	313	14/14	100.0	78.5-100.0	298/299	99.7	98.1-99.9
Coronavirus NL63	312	24/24	100.0	86.2-100.0	286/288	99.3	97.5-99.8
Coronavirus OC43	310	28/28	100.0	87.9-100.0	282/282	100.0	98.6-100.0
Human Metapneumovirus	313	2/2	100.0	34.2-100.0	311/311	100.0	98.7-100.0
Rhinovirus/Enterovirus	313	44/49	89.8	78.2-95.5	254/264	96.2	92.3-97.9
Influenza A	313	17/17	100.0	81.5-100.0	296/296	100.0	98.7-100.0
Influenza A H1	313	0/0	NA	NA	313/313	100.0	98.8-100.0
Influenza A H1N1/2009	312	7/8	87.5	52.9-97.8	304/304	100.0	98.9-100.0
Influenza A H3	313	8/8	100.0	67.5-100.0	305/305	100.0	98.8-100.0
Influenza B	313	1/1	100.0	20.7-100.0	312/312	100.0	98.8-100.0
Parainfluenza Virus 1	307	40/40	100.0	91.2-100.0	267/267	100.0	98.8-100.0
Parainfluenza Virus 2	312	3/3	100.0	100.0	309/309	100.0	98.8-100.0
Parainfluenza Virus 3	313	1/4	25.0	4.6-69.9	309/309	100.0	98.8-100.0
Parainfluenza Virus 4	302	22/24	91.7	74.2-97.7	278/278	100.0	98.6-100.0
Respiratory Syncytial Virus	313	11/12	91.7	64.6-98.5	300/301	99.7	98.4-99.9
Bacteria							
<i>Bordetella pertussis</i>	294	33/33	100.0	89.6-100.0	261/261	100.0	98.5-100.0
<i>Chlamydia pneumoniae</i>	311	54/61	88.5	78.2-94.3	250/250	100.0	98.5-100.0
<i>Mycoplasma pneumoniae</i>	313	25/25	100.0	86.7-100.0	287/288	99.7	98.1-99.9

486 ^aThese data are presented based on comparator assay (BioFire FilmArray Respiratory Panel
487 version 1.7) only and do not reflect any discordant analysis.

488 ^bIn instances where the internal control failed and was not resolved upon repeat, any target that
489 was “Detected” was maintained within the data set and used in performance calculations. All
490 targets that were not detected were considered failed and excluded from the data analysis
491 therefore the final “N” will vary by analyte.

492

493 **TABLE 4** Results of Discrepant Investigation for QIAstat-Dx Respiratory Panel (QDRP) -
 494 Prospective and Retrospective Specimens

495

Result Disposition based on initial testing versus comparator	False Negatives ^a			False Positives		
	QDRP Result	Discrepant Investigation Outcome:		QDRP Result	Discrepant Investigation Outcome:	
		QDRP confirmed ^b (TN)	QDRP unconfirmed (FN)		QDRP confirmed ^b (TP)	QDRP unconfirmed (FP)
Analyte	Total FN			Total FP		
Viruses						
Adenovirus ^c	4	1	3	23	9	14
Coronavirus 229E ^d	2	0	2	0	-	-
Coronavirus HKU1	1	1	0	8	0	8
Coronavirus NL63 ^e	7	0	7	4	1	3
Coronavirus OC43 ^f	3	3	0	4	3	1
Human Metapneumovirus	7	3	4	5	3	2
Rhinovirus/Enterovirus ^g	31	9	22	46	18	28
Influenza A ^h	2	1	1	9	3	6
Influenza A H1 ⁱ	1	0	1	0	-	-
Influenza A H1/2009	2	0	2	5	3	2
Influenza A H3	1	0	1	7	7	0
Influenza B ^j	7	0	7	1	1	0
Parainfluenza Virus 1	1	1	0	3	3	0
Parainfluenza Virus 2	0	-	-	0	-	-
Parainfluenza Virus 3	5	2	3	5	3	2
Parainfluenza Virus 4	2	1	1	2	2	0
Respiratory Syncytial Virus	9	7	2	6	3	3
Bacteria						
<i>Bordetella pertussis</i> ^k	0	-	--	6	1	5
<i>Chlamydia pneumoniae</i> ^l	7	1	6	1	1	0
<i>Mycoplasma pneumoniae</i> ^m	0	-	--	6	1	5
Total	92	30	62	141	62	79

496

497 ^a Result disposition based on initial testing with QDRP versus comparator testing with BioFire

498 FilmArray Respiratory Panel version 1.7.

499 ^bQIAstat-Dx RP confirmed, the results of discrepant analysis supported the original QIAstat-Dx

500 Respiratory Panel result as true negative or true positive. QIAstat-Dx RP unconfirmed, the

501 results of discrepant analysis did not support the original QIAstat-Dx Respiratory Panel result

502 and result considered false negative or false positive. TN, true negative, FN, false negative; TP,
503 true positive; FP, false positive.

504 ^cTwo (2) FP adenovirus specimen did not undergo discordant analysis and were considered
505 unconfirmed FP.

506 ^dTwo (2) FN Coronavirus E229 specimen did not undergo discordant analysis and were
507 considered unconfirmed FN.

508 ^eTwo (2) FP Coronavirus NL63 specimen did not undergo discordant analysis and were
509 considered unconfirmed FP.

510 ^fOne (1) FN Coronavirus OC43 specimen did not undergo discordant analysis and was
511 considered unconfirmed FN.

512 ^gThree (3) FN Rhinovirus/Enterovirus specimen did not undergo discordant analysis and were
513 considered unconfirmed FN.

514 ^hThree (3) FP Influenza A samples were not available for discrepancy testing and were
515 considered unconfirmed FP.

516 ⁱNon-2009 H1 has not been in circulation since being replaced by the 2009 H1 and thus the
517 discrepancy test result for the FN 2009-H1 sample is likely false

518 ^jOne (1) FN Influenza B sample was not available for discrepancy testing and was considered
519 unconfirmed FN.

520 ^kOne (1) FP *Bordetella pertussis* sample was not available for discrepancy testing and was
521 considered unconfirmed FP.

522 ^lTwo (2) FN *Chlamydia pneumoniae* samples were not available for discrepancy testing and
523 were considered unconfirmed FN.

524 ^mOne (1) FP *Mycoplasma pneumoniae* sample was not available for discrepancy testing and
525 another FP (1) sample did not produce a valid result with the discrepancy method; both FP
526 results were considered unconfirmed FP.

527

528

529

530

531



① Load sample



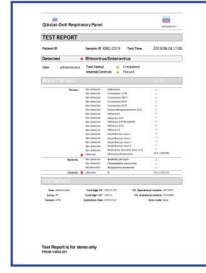
② Scan barcode



③ Insert cartridge



④ Start run



⑤ Obtain report