ONLINE SUPPLEMENT

Diagnosis of Primary Ciliary Dyskinesia: An Official ATS Clinical Practice Guideline

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Figure E1.1: PRISMA Flow diagram for Question 1

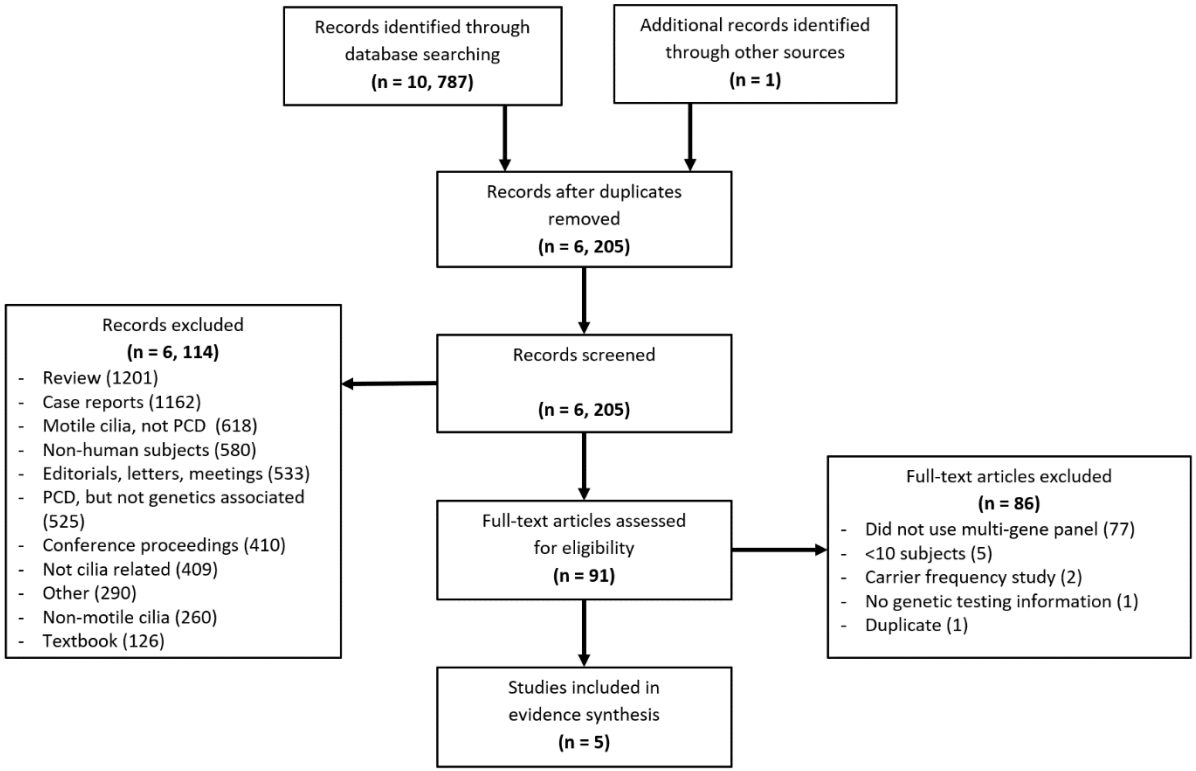
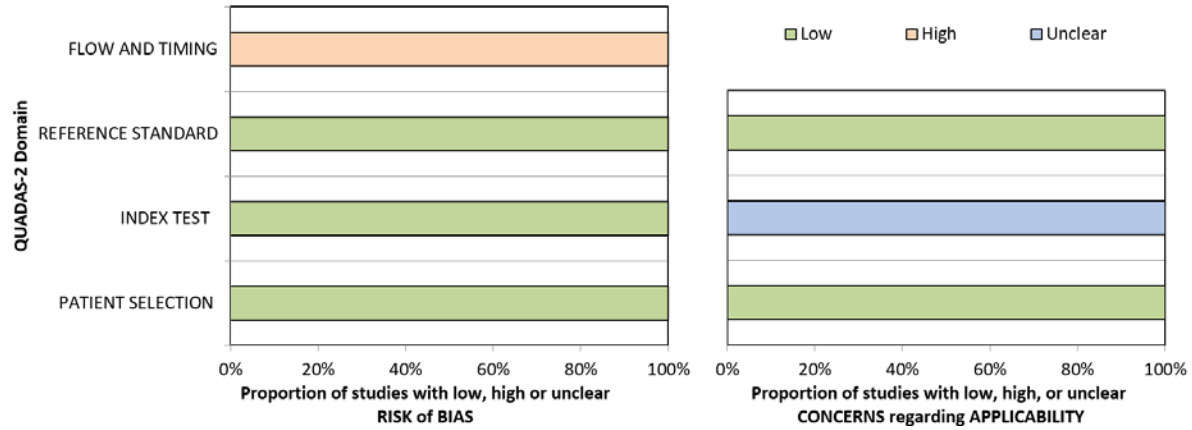


Figure E1.2: Quality assessment of individual studies with QUADAS-2 for Question 1



	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Leigh 2017	Low	Low	Low	High

	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Leigh 2017	Low	Unclear	Low

1. Leigh MW, Ferkol TW, Davis SD, Lee HS, Rosenfeld M, Dell SD, Sagel SD, Milla C, Olivier KN, Sullivan KM, Zariwala MA, Pittman JE, Shapiro AJ, Carson JL, Krischer J, Hazucha MJ, Knowles MR. Clinical Features and Associated Likelihood of Primary Ciliary Dyskinesia in Children and Adolescents. *Ann Am Thorac Soc* 2016; 13: 1305-1313.

Figure E1.3: Forest plot of included article

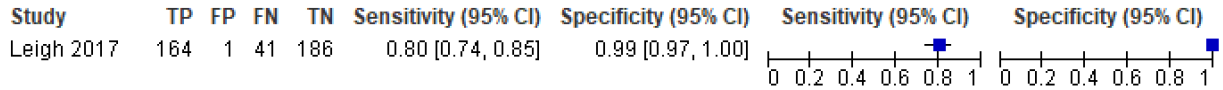


Table E1.1: Summary of findings table for Question 1

Sensitivity	0.80 (95% CI: 0.74 to 0.85)
Specificity	1.00 (95% CI: 0.97 to 1.00)

Prevalence	35%(1)

Outcome	№ of studies (№ of patients)	Study design	Factors that may decrease quality of evidence					Effect per 100 patients tested	Test accuracy QoE	Importance
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 35%		
True positives (patients with PCD)	1 study 205 patients(1)	cross-sectional (cohort type accuracy study)	serious [a]	not serious	not applicable	not serious	none	28 (26 to 30)	⊕⊕⊕◯ MODERATE	CRITICAL
False negatives (patients incorrectly classified as not having PCD)								7 (5 to 9)		CRITICAL
True negatives (patients without PCD)	1 study 187 patients(1)	cross-sectional (cohort type accuracy study)	serious [a]	not serious	not applicable	not serious	none	65 (63 to 65)	⊕⊕⊕◯ MODERATE	IMPORTANT
False positives (patients incorrectly classified as having PCD)								0 (0 to 2)		IMPORTANT

Table E1.2. Evidence to Decision Table – Question 1

Should an extended genetic panel (testing >12 genes) be used as a diagnostic test in adult and pediatric patients with a high probability of having PCD (as replacement of reference standards of classic TEM structural ciliary defect AND/OR standard genetic panels testing for mutations in ≤12 genes associated with PCD)?		
POPULATION:	Patients with a high pre-test probability	**BACKGROUND:** PCD is a genetically heterogeneous and predominantly autosomal recessive disorder caused by biallelic pathogenic mutations in one of the many identified PCD causative genes (39 to date). Each PCD diagnostic test carries limitations, and those tests dependent on respiratory mucosal (ciliary) biopsy (TEM, CBF, HSVM) are encumbered by the need for on-site, high-quality specimen sampling, processing and analysis. The widespread lack of local expertise and resources in ciliary biopsy testing has made molecular genetic testing an attractive alternative. Genetic testing for a Mendelian disease has the added value of procuring inherently high specificity, however sensitivity may be expected to be lacking in a genetically heterogeneous disease such as PCD. In a comprehensive review of the PCD literature in 2015, Zariwala and colleagues demonstrated that more than 50% of PCD patients possess two pathogenic mutations in trans in a known PCD causative gene (25). However the sensitivity of genetic testing is anticipated to increase as commercial diagnostic panels incorporate novel identified PCD genes. Since genetic testing for PCD is already available in CLIA certified laboratories and costs have been decreasing, the impetus to consider molecular genetic testing as a first-line diagnostic test for PCD is increasing.
INTERVENTION:	Extended panel genetic testing	
PURPOSE OF THE TEST:	Diagnosis of PCD	
LINKED TREATMENTS:	Targeted pulmonary/ENT care in a PCD specialized center in patients with confirmed PCD or further investigations for other potentially treatable diseases in patients with negative testing for PCD	
ANTICIPATED OUTCOMES:	Premature death, need for lung transplant, rapid deterioration of pulmonary function, restriction in physical functioning/activity, development of bronchiectasis, deterioration of overall quality of life, recurrent sinopulmonary exacerbations, recurrent hospitalizations, hearing loss or speech delay, recurrent antibiotics use, need for ear tube placement, need for sinus surgery, infertility, depression/anxiety and side effects of repeat testing, absenteeism, poor social functioning, resources use	
SETTING:	Outpatient setting	
PERSPECTIVE:	Clinical recommendation from an individual perspective	

	JUDGMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
PROBLEM	**Is the problem a priority?** ○ No ○ Probably no ○ Probably yes ○ **Yes** ○ Varies ○ Don't know	A growing number of clinical centers across North America employ genetic testing to diagnose PCD. In the past several years, commercially available PCD genetic testing has greatly expanded, with multiple companies offering NGS panels. Initially, these panels showed poor sensitivity as they investigated mutations in ≤12 PCD-causing genes, but current NGS panels now test for mutations >30 known PCD genes. Genetic testing is attractive as it is highly feasible, with local blood draws and central sample processing in commercial laboratories. (1, 2). Although commercial PCD genetic testing is widely available in North America, payment for this testing can be difficult to obtain through insurance and governmental coverages. The access to genetic testing in Europe is even more limited due to similar payment issues. While this test is highly feasible (requiring only peripheral venipuncture) and does not require physical access to specialized centers, results can be uninformative with frequently encountered variants of unknown significance (3-8). Genetic testing also has limitations in PCD diagnosis, as the number of PCD-causing genes continues to grow rapidly and a complete panel does not exist (1). Currently, PCD-causing genetic mutations are only known in approximately 70% of all proven PCD cases(2). Electron microscopy ciliary analysis is difficult to perform correctly outside of highly experienced centers, with some major academic centers suffering from poor feasibility for this complex test (20-40% of samples are inconclusive or lack sufficient material for analysis) (9-11). In experienced research centers, this feasibility is greatly improved (12). Other centers misinterpret secondary ciliary changes on TEM as primary, disease-causing defects, leading to false positive results (13, 14). TEM analysis is also costly (approximately $1000 USD per test), and at least 10-20% of patients require repeat TEM testing to confirm their defects (11, 15). Some centers prefer lower airway samples for their TEM analysis (as opposed to nasal biopsies), requiring a general anesthesia in most pediatric patients (11). Lastly, TEM will be normal in approximately 30% of PCD cases confirmed by other testing (genetics, HSVM, immunofluorescent staining)(2, 10).	

<table>
<tr>
<td>TEST ACCURACY</td>
<td>How accurate is the test?
○ Very inaccurate
○ Inaccurate
○ Accurate
○ Very accurate

○ Varies
○ Don't know</td>
<td>Using an extended genetic PCD panel against the combined reference standard of TEM analysis and/or a standard genetic panel (≤12 genes), one study reported a sensitivity of 80% (95% CI 74.0-84.9) and specificity was 99.5% (95% CI 97.0-99.9), in a population with a pre-test probability of 35%.
<table>
<tr><th>Test results</th><th>Importance</th><th>Effects per 100 patients tested (prevalence = 35%)</th><th>Quality of evidence</th></tr>
<tr><td>TP</td><td>Critical</td><td>28 (26 to 30)</td><td rowspan="2">⊕⊕⊕◯
MODERATE</td></tr>
<tr><td>FN</td><td>Critical</td><td>7 (5 to 9)</td></tr>
<tr><td>TN</td><td>Important</td><td>65 (63 to 65)</td><td rowspan="2">⊕⊕⊕◯
MODERATE</td></tr>
<tr><td>FP</td><td>Important</td><td>0 (0 to 2)</td></tr>
</table>
</td>
<td>The panel also considered that the sensitivity may even be higher, as the reference standard of TEM performs much worst outside of specialized PCD research centers. Furthermore, newly discovered PCD-causing genes, resulting in normal or non-diagnostic TEM studies, are now detected on extended genetic PCD panels. As more PCD-causing gene mutations are discovered and included on current genetic panels, the diagnostic accuracy of extended genetic panel will likely improve even further for PCD.</td>
</tr>
</table>

	Index test + (2 mutations in PCD gene on extended panel testing)	Index test – (<2 disease-causing mutations on extended panel testing)
PCD +	TRUE POSITIVES -Referral to a PCD specialized center -Rapid cessation of repeat testing, thus avoid unnecessary supplementary costs and anxiety over awaiting confirmation of PCD diagnosis -PCD targeted pulmonary and ENT therapies with probable clinical improvement	FALSE NEGATIVES ** -May still have PCD as not all disease-causing mutations and genes are currently known -Discharge from a PCD specialized center (*diagnosis of PCD will likely be missed*) -Unnecessary investigation for other diseases -Unnecessary supplementary costs and anxiety over awaiting diagnosis -No PCD targeted pulmonary and ENT care, and may receive other non-PCD cares with risks (e.g. IVIG with blood product exposures, lobectomy)
PCD -	FALSE POSITIVES * -Referral to a PCD specialized center (*diagnosis of the true disease will likely be delayed*) -PCD targeted pulmonary and ENT care with possible clinical improvement regardless of the cause of chronic lung disease. -No specific therapy for the true underlying disease, if it exists (e.g. IVIG for immunodeficiency)	TRUE NEGATIVES -Discharge from a PCD specialized center -Investigation for other potentially treatable diseases (such as immunodeficiency) -Rapid cessation of repeat testing, thus avoid unnecessary supplementary costs and anxiety over awaiting information of PCD diagnosis

Nevertheless, case series of consecutively identified PCD patients shows a clear proportional increase in sensitivity as the number of genes included in the panel

		increases. In the Kim and Marshall papers, sensitivity increased from 71.9% (12 genes)(8) to 94.7% (32 + genes)(6) in the same cohort.	
DESIRABLE EFFECTS	**How substantial are the desirable anticipated effects?** o Trivial o Small o **Moderate** o Large o Varies o Don't know		*The panel considered that the undesirable downstream consequences of false positive results are difficult to assess and thus uncertain for 2 main reasons: 1) false positive results could still be PCD since ongoing studies are showing that the references standards of TEM and genetic testing lack sensitivity to detect PCD (i.e. new genetic variants are discovered each year) 2) great heterogeneity in the non-PCD true underlying disease thus the expected effects of the PCD targeted pulmonary and ENT therapies. However, these false positive diagnoses would likely receive airway clearance therapy, which would be of clinical benefit in any chronic suppurative lung disease, regardless of the underlying cause. **The panel considered that the undesirable downstream consequences of false negative results difficult to assess and thus uncertain for 2 main reasons: 1) the effect could be have been underestimated since the studies assessing the impact of delayed diagnosis were not recently performed,
UNDESIRABLE EFFECTS	**How substantial are the undesirable anticipated effects?** o Large o Moderate o **Small** o Trivial o Varies o Don't know		

			and the standard of care has greatly improved (as well as the patient outcomes), 2) the effect could have been overestimated since older age at PCD diagnosis (usually correlated with delayed diagnosis) is associated with distrust in medical community, with less improvement in the St George's Respiratory Questionnaire scores, worsened long-term compliance with PCD treatment regimens (16) and ultimately, with worse outcomes (increased rates of respiratory cultures positive for *Pseudomonas aeruginosa* infection (17), which causes worse outcomes in similar respiratory diseases (18), increased rates of medical and surgical complications, including nasal polyposis, hemoptysis, and lobectomy surgery, all of which can cause significant morbidity and even mortality.
CERTAINTY OF THE EVIDENCE OF TEST ACCURACY	**What is the overall certainty of the evidence of test accuracy?** o Very low o Low o **Moderate** o High o No included studies	Risk of bias of included studies led to rating down the certainty in the evidence. Detailed judgment in provided in the evidence tables.	

CERTAINTY OF THE EVIDENCE OF TEST'S EFFECTS	**What is the overall certainty of the evidence for any critical or important direct benefits, adverse effects or burden of the test?** ○ Very low ○ Low ○ Moderate ○ High ○ **No included studies**	No direct evidence for critical or important direct benefits, adverse effects or burden of the test (i.e. side effects of repeat testing and anxiety related to delayed diagnosis) was considered here.	The panel assumed that: 1) Extended panel PCD genetic testing can be performed via phlebotomy locally (causing only minor discomfort), but results are delayed for weeks to months and can be non-diagnostic, with variants of unknown significance (1) requiring other PCD diagnostic tests. 2) TEM analysis sometimes requires patients to travel to experienced PCD centers, can take weeks to months to produce results, gives non-diagnostic results requiring repeat biopsies (reported inconclusive results rates vary between 20% and 42% in experienced centers performing sampling under optimal conditions (9-11, 15, 19)), and complications of biopsy are minimal (mild discomfort, possibly mild bleeding)(20, 21). If extended panel genetic testing replaces TEM and/or standard panel genetic testing, patients may not require repeat mucosal biopsies for TEM studies, thus

			should ultimately reduce unnecessary costs associated with repeat TEM testing and anxiety related to delayed diagnosis.
CERTAINTY OF THE EVIDENCE OF MANAGEMENT'S EFFECTS	**What is the overall certainty of the evidence of effects of the management that is guided by the test results?** ○ Very low ○ **Low** ○ Moderate ○ High ○ No included studies	No direct evidence comparing PCD targeted pulmonary and ENT care versus no treatment was considered since these treatments consist of a bundle of different supportive therapies which are usually at least partially started for symptom relief. Nevertheless, longitudinal PCD studies show that patient using long term standard PCD regimens experienced less decline in lung function than patients left undiagnosed and thus untreated (22-24). Referral of pediatric patients to a PCD center of excellence for long-term therapies may also improve lung function and nutrition (25). Furthermore, later diagnosis (in adulthood) of PCD might be linked to worsened long-term pulmonary outcomes (22). Other individual interventions were occasionally studied but could not be pooled due to the heterogeneity of interventions and/or comparators for each critical outcome. For instance, children with PCD and chronic otitis media with effusion show marked improvements in hearing after surgical placement of ventilation tubes versus medical therapy alone (26, 27). Aggressive surgical management of chronic rhinosinusitis in PCD patients also provides significant symptom relief (28). Regular airway clearance also shows improvements in lung function in one small cross-over RCT (29).	The panel considered that standard PCD therapies are likely more efficient than what is currently reported, but equipoise would preclude studying the natural evolution of the disease without minimal intervention.
CERTAINTY OF THE EVIDENCE OF TEST RESULT/MANAGEMENT	**How certain is the link between test results and management decisions?** ○ **Very low** ○ Low ○ Moderate ○ High ○ No included studies	Observational studies showed that PCD patients will promptly begin standard therapies for PCD, including daily airway clearance, sputum culture surveillance, otolaryngology care, and aggressive use of antibiotics for respiratory infections (30, 31). Nevertheless, these therapies may be suboptimal outside of PCD specialized centers. Furthermore, erratic long-term compliance with PCD treatment regimens, especially in older patients at diagnosis (16), increases uncertainty regarding the link between testing and treatment.	The panel confirms that in clinical practice a positive diagnostic for PCD will almost certainly lead to the start of chronic therapies if patient is referred to a PCD specialized center.

<table>
<tr>
<td>CERTAINTY OF EFFECTS</td>
<td>What is the overall certainty of the evidence of effects of the test?
○ Very low
○ Low
○ Moderate
○ High

○ No included studies</td>
<td>The overall certainty of the evidence of the effects of testing and subsequent management decisions on patient-important outcomes is limited by the very low certainty regarding the link between tests results and management decisions and the low certainty of the effects of the management guided by the test results.</td>
<td></td>
</tr>
<tr>
<td>VALUES</td>
<td>Is there important uncertainty about or variability in how much people value the main outcomes?
○ Important uncertainty or variability
○ Possibly important uncertainty or variability
○ Probably no important uncertainty or variability
○ No important uncertainty or variability</td>
<td>There are also numerous publications addressing the stress created in patients surrounding their difficulty obtaining a proper PCD diagnosis. Indeed, uncertainty surrounding PCD diagnosis has been linked to poor psychosocial outcomes (32, 33). Several PCD patients and family representatives of PCD patients sat on this committee, and they repeatedly voiced their frustration with poor quality diagnostic testing and ambiguous diagnostic results. To these stakeholders, accurate PCD diagnosis is of the utmost importance and is the first step towards successfully managing their PCD in the long-term. Research has demonstrated that other PCD patients feel the same as our patient representatives, with many harboring distrust of the medical system over the uncertainty surrounding their PCD diagnosis. Patients also report feeling stigmatized and embarrassed due to long-term uncertainty over their PCD diagnosis (34). Patients with accurate genetic testing can also use this information for family planning through genetic counseling and possibly for prognosis of long-term disease progression, as some specific PCD mutations may result in worse or milder lung disease and nutrition (35, 36).</td>
<td>The panel which included patients' representatives made the following assumptions about the patient-important outcomes:

<table>
<tr><th>Outcomes</th><th>Relative importance</th></tr>
<tr><td>Premature death</td><td>CRITICAL</td></tr>
<tr><td>Need for lung transplant</td><td>CRITICAL</td></tr>
<tr><td>Lobectomy</td><td>CRITICAL</td></tr>
<tr><td>Rapid deterioration of pulmonary function</td><td>CRITICAL</td></tr>
<tr><td>Restriction in physical functioning/activity</td><td>CRITICAL</td></tr>
<tr><td>Development of bronchiectasies</td><td>CRITICAL</td></tr>
<tr><td>Deterioration of quality of life</td><td>CRITICAL</td></tr>
<tr><td>Recurrent sinopulmonary exacerbations</td><td>CRITICAL</td></tr>
<tr><td>Recurrent hospitalisations</td><td>CRITICAL</td></tr>
</table>
</td>
</tr>
</table>

			Hearing loss or speech delay	CRITICAL
			Recurrent antibiotics use	IMPORTANT
			Need for ear tube placement	IMPORTANT
			Need for sinus surgery	IMPORTANT
			Infertility	IMPORTANT
			Anxiety related to delayed diagnosis	IMPORTANT
			Side effects of repeat testing	IMPORTANT
			Absenteeism	IMPORTANT
			Poor social functioning	IMPORTANT
			Resources use	IMPORTANT
BALANCE OF EFFECTS	**Does the balance between desirable and undesirable effects favor the intervention or the comparison?** ○ Favors the comparison ○ Probably favors the comparison ○ Does not favor either the intervention or the comparison ○ **Probably favors the intervention** ○ Favors the intervention ○ Varies ○ Don't know	The balance of direct desirable/undesirable effects favors the index test over the reference standard. False negative results, which are of critical importance in this analysis, are estimated to be similar in frequency with the extended genetic panel and the reference standard. Thus, the balance of downstream consequences does not favor either the index test or reference standard.		

<table>
<tr>
<td>RESOURCES REQUIRED</td>
<td>How large are the resource requirements (costs)?
○ Large costs
○ Moderate costs
○ Negligible costs and savings
○ Moderate savings
○ Large savings

○ Varies
○ Don't know</td>
<td>
<table>
<tr><th></th><th>TEM*</th><th>Standard genetic panel*</th><th>Extended genetic panel*</th></tr>
<tr><td>St Louis, Missouri, USA</td><td>$1,520</td><td>$950</td><td>$950 (37)</td></tr>
<tr><td>Israel</td><td>$1,000</td><td>not provided</td><td>not provided</td></tr>
<tr><td>Southampton, UK</td><td>$730</td><td>not provided</td><td>not provided</td></tr>
<tr><td>Montreal, Canada</td><td>$550</td><td>$950</td><td>$950</td></tr>
<tr><td>Denver, Colorado, USA</td><td>$715</td><td>$950</td><td>$950</td></tr>
<tr><td>Meunster, Germany</td><td>$750</td><td>not provided</td><td>$2,900</td></tr>
</table>
All prices are presented in US dollars.
*Assuming that the baseline equipment/device is already available within the hospitals offering the tests.
</td>
<td>The cost for commercial genetic panels in North America is similar to the costs of TEM analysis, but the poor feasibility of TEM means that this test is sometimes repeated at additional cost to the patient. Most academic sites already own the necessary laboratory equipment for ciliary TEM and many sites send their ciliary biopsies to third party sites for TEM processing and analysis. Genetic testing does not require institutions to purchase any start-up materials, as most sample processing and analysis is performed in commercial laboratories. Both extended panel genetic testing and TEM ciliary analysis are approved for clinical use in the USA.</td>
</tr>
<tr>
<td>CERTAINTY OF EVIDENCE OF REQUIRED RESOURCES</td>
<td>What is the certainty of the evidence of resource requirements (costs)?
○ Very low
○ Low
○ Moderate
○ High

○ No included studies</td>
<td>All cost information was obtained from international expert PCD centers, through personal communications with center directors.</td>
<td></td>
</tr>
<tr>
<td>COST EFFECTIVENESS</td>
<td>Does the cost-effectiveness of the intervention favor the intervention or the comparison?</td>
<td>No research evidence was identified.</td>
<td>The cost-effectiveness of clinical TEM and extended panel genetic testing are roughly equivalent. While insurance</td>
</tr>
</table>

	○ Favors the comparison ○ Probably favors the comparison ○ Does not favor either the intervention or the comparison ○ **Probably favors the intervention** ○ Favors the intervention ○ Varies ○ No included studies		companies in North America usually readily cover TEM analysis, they sometimes refuse to cover genetic testing. Government supported health programs in Europe do cover TEM analysis but do not pay for genetic testing.
EQUITY	**What would be the impact on health equity?** ○ Reduced ○ Probably reduced ○ Probably no impact ○ **Probably increased** ○ Increased ○ Varies ○ Don't know	No research evidence was identified.	Extended panel genetic testing does not require travel to a specialized center, whereas TEM testing often requires travel to a center that can at least obtain a mucosal biopsy, even if this sample is then sent to a third-party service for processing and interpretation. If third-party processing/interpretation is unavailable, patients will need to travel to a tertiary care center for mucosal biopsy and TEM analysis. The financial implications are unclear due to variability in charges and reimbursements for different procedures.

QU	**Is the intervention acceptable to key stakeholders?** ○ No ○ Probably no ○ Probably yes ○ **Yes** ○ Varies ○ Don't know	No research evidence was identified.	Genetic testing is easy to perform for patients, does not require travel, is less painful to obtain than a mucosal biopsy, and often does not require repeat sample acquisition. Costs to the consumer are equivalent to TEM analysis, and as more PCD genes are include in extended genetic panels, the diagnostic accuracy will continue to improve. Thus, PCD patients and families of PCD patients on this committee strongly approved of this intervention.
FEASIBILITY	**Is the intervention feasible to implement?** ○ No ○ Probably no ○ Probably yes ○ **Yes** ○ Varies ○ Don't know	No research evidence was identified.	Commercial entities throughout North America offer extended panel genetic testing for PCD. Thus, implementation widespread genetic testing would be straightforward.

Summary of judgments – Question 1

	JUDGMENT							IMPLICATIONS
PROBLEM	No	Probably no	Probably yes	**Yes**		Varies	Don't know	
TEST ACCURACY	Very inaccurate	Inaccurate	**Accurate**	Very accurate		Varies	Don't know	
DESIRABLE EFFECTS	Trivial	Small	**Moderate**	Large		Varies	Don't know	
UNDESIRABLE EFFECTS	Large	Moderate	**Small**	Trivial		Varies	Don't know	
CERTAINTY OF THE EVIDENCE OF TEST ACCURACY	Very low	Low	**Moderate**	High			No included studies	
CERTAINTY OF THE EVIDENCE OF TEST'S EFFECTS	Very low	Low	Moderate	High			**No included studies**	
CERTAINTY OF THE EVIDENCE OF MANAGEMENT'S EFFECTS	Very low	**Low**	Moderate	High			No included studies	
CERTAINTY OF THE EVIDENCE OF TEST RESULT/MANAGEMENT	**Very low**	Low	Moderate	High			No included studies	
CERTAINTY OF EFFECTS	**Very low**	Low	Moderate	High			No included studies	
VALUES	Important uncertainty or variability	Possibly important uncertainty or variability	Probably no important uncertainty or variability	**No important uncertainty or variability**				

	JUDGMENT							IMPLICATIONS
BALANCE OF EFFECTS	Favors the comparison	Probably favors the comparison	Does not favor either the intervention or the comparison	**Probably favors the intervention**	Favors the intervention	Varies	Don't know	
RESOURCES REQUIRED	Large costs	Moderate costs	**Negligible costs and savings**	Moderate savings	Large savings	Varies	Don't know	
CERTAINTY OF EVIDENCE OF REQUIRED RESOURCES	Very low	Low	Moderate	High			**No included studies**	
COST EFFECTIVENESS	Favors the comparison	Probably favors the comparison	Does not favor either the intervention or the comparison	**Probably favors the intervention**	Favors the intervention	Varies	No included studies	
EQUITY	Reduced	Probably reduced	Probably no impact	**Probably increased**	Increased	Varies	Don't know	
ACCEPTABILITY	No	Probably no	Probably yes	**Yes**		Varies	Don't know	
FEASIBILITY	No	Probably no	Probably yes	**Yes**		Varies	Don't know	

Conclusions – Question 1

Should an extended genetic panel (testing >12 genes) be used as a diagnostic test in adult and pediatric patients with a high probability of having PCD (as replacement of reference standards of classic TEM structural ciliary defect AND/OR standard genetic panels testing for mutations in ≤12 genes associated with PCD)?

TYPE OF RECOMMENDATION	Strong recommendation against the intervention	Conditional recommendation against the intervention	Conditional recommendation for either the intervention or the comparison	**Conditional recommendation for the intervention**	Strong recommendation for the intervention
RECOMMENDATION	We suggest using extended genetic panel testing >12 PCD genes to diagnose PCD in patients who have a high probability of having PCD on the basis of compatible clinical phenotype, compared to the references standards of classic TEM ultrastructural ciliary defect and/or standard genetic panels testing for mutations in ≤12 genes associated with PCD				
JUSTIFICATION	The direct desirable consequences of using extended genetic panel testing instead of the reference standards outweigh the undesirable consequences. The overall impact of avoiding direct costs, complications and burden of repeat testing justified using extended genetic panel testing as a replacement to the reference standards. The overall rate of false negatives (which was considered critical) and false positives were small and thus the downstream consequences were considered similar between the two test strategies. The overall impact of avoiding direct costs, complications and burden of repeat testing justified using this extended panel genetic testing as a replacement to reference standards. Furthermore, extended genetic panel testing was probably cost-effective, more equitable as well as clearly acceptable to key stakeholders and feasible to implement. Lastly, patients with accurate genetic testing can also use this information for family planning through genetic counseling and possibly for prognosis of long-term disease progression, as some specific PCD mutations may result in worse or milder lung disease and nutrition (35, 36)				
SUBGROUP CONSIDERATIONS	If extended panel genetic testing is negative in a patient at high probability of having PCD (very robust clinical phenotype), then further diagnostic testing should be done to confirm or refute a diagnosis of PCD.				
IMPLEMENTATION CONSIDERATIONS	Implementation should be straightforward as commercial companies already offer extended panel PCD genetic testing across North America. European and international health plans will need to adopt payment policies for genetic testing on their populations.				
MONITORING AND EVALUATION	Companies offering extended panel genetic testing must keep their collection of analyzed genes and mutations up to date, as new PCD-causing mutations and genes are discovered frequently. References for variants of unknown significance are essential for proper interpretation of equivocal results and local genetic services in clinical centers may be required for inconclusive results.				

RESEARCH PRIORITIES	Research trials evaluating all possible strategies and patient-important outcomes should be performed, specifically including trials of extended genetic panel testing versus other PCD diagnostic modalities. Additionally, studies on genetic discovery of novel PCD-causing genes and mutations are necessary to improve diagnostic accuracy of extended genetic panel testing.

References

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Figure E2.1 - PRISMA Flow diagram for question 2

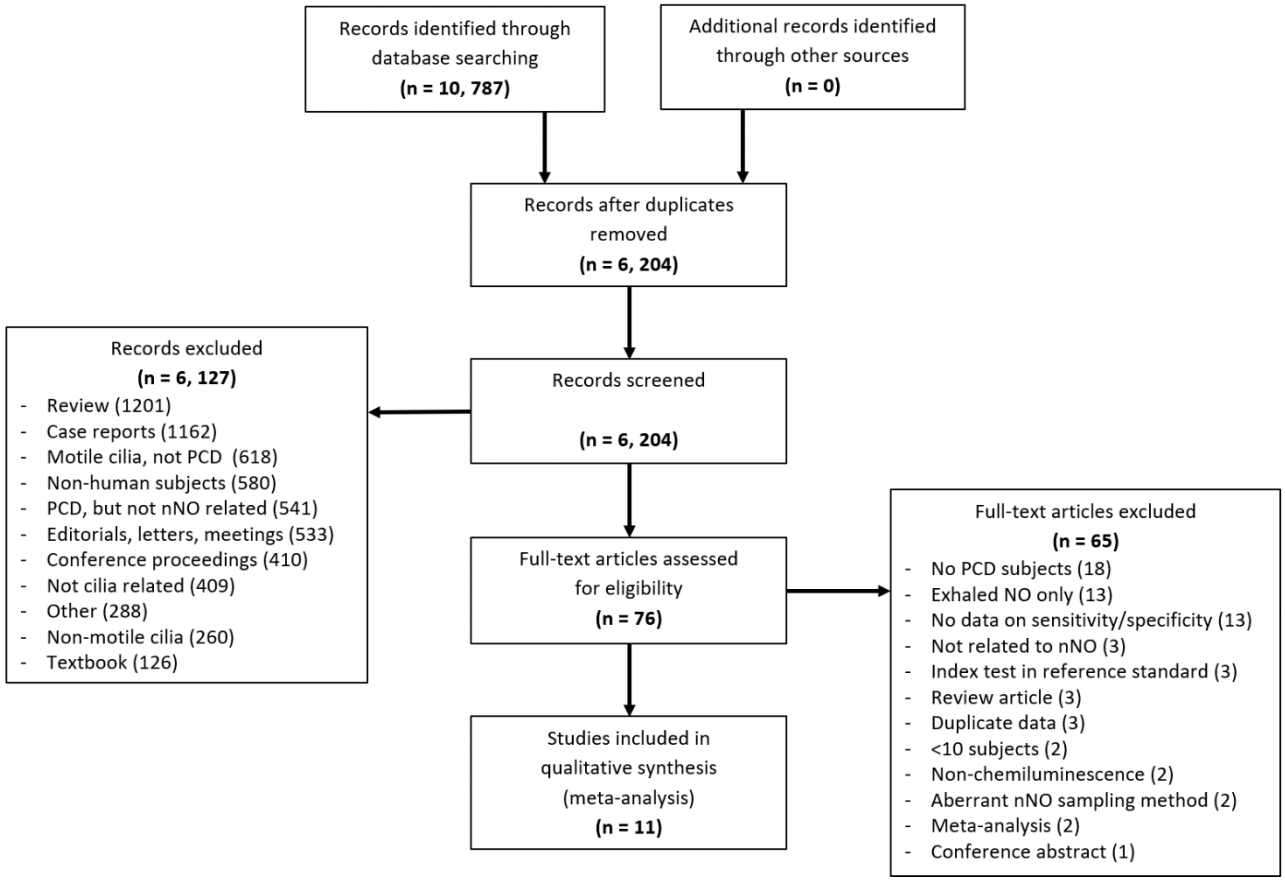
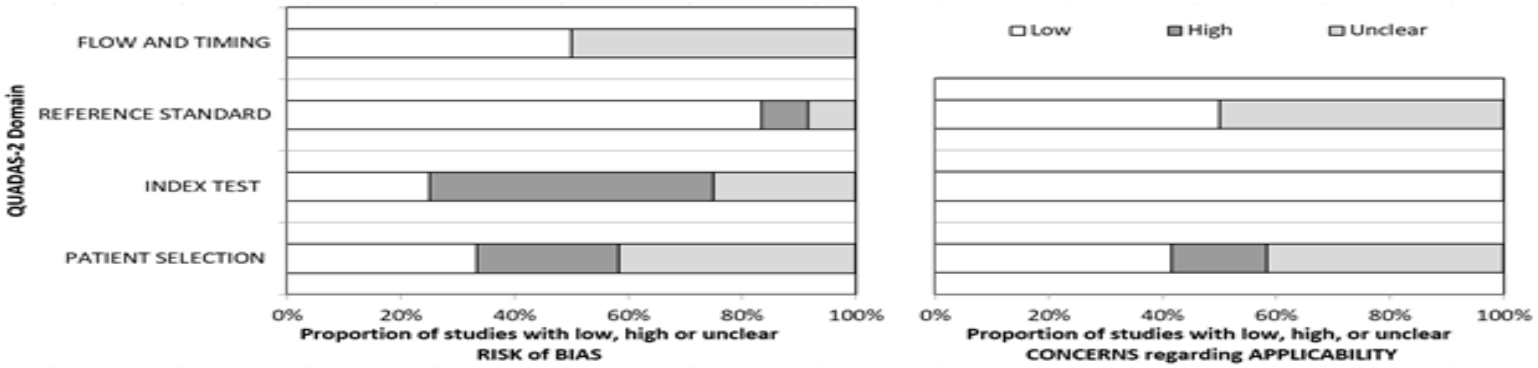


Figure E2.2 - Assessment of validity of individual studies with QUADAS-2 tool for the 12 included studies for Question 2

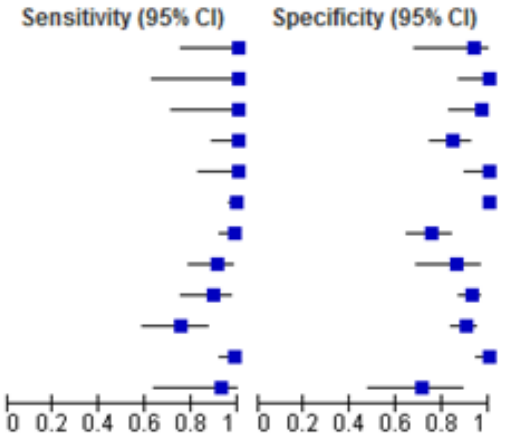


	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Beydon 2015	Low	High	Unclear	Low
Boon 2014	Unclear	Low	Low	Unclear
Harris 2014	Unclear	High	Low	Unclear
Leigh 2013 (leading site)	Unclear	Unclear	Low	Unclear
Leigh 2013 (other sites)	Low	Low	Low	Low
Mateos Coral 2011	Unclear	High	Low	Unclear
Noone 2004	High	Unclear	Low	Unclear
Papon 2012	Low	Low	Low	Low
Piacentini 2008	High	High	Low	Low
Pfierri 2011	Low	Unclear	Low	Low
Santamaria 2008	High	High	Low	Low
Wodehouse 2003	Unclear	High	High	Unclear

	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Beydon 2015	Low	Low	Low
Boon 2014	Unclear	Low	Low
Harris 2014	Unclear	Low	Low
Leigh 2013 (leading site)	Unclear	Low	Low
Leigh 2013 (other sites)	Low	Low	Low
Mateos Coral 2011	Unclear	Low	Unclear
Noone 2004	High	Low	Unclear
Papon 2012	Low	Low	Unclear
Piacentini 2008	High	Low	Unclear
Pfierri 2011	Low	Low	Low
Santamaria 2008	Unclear	Low	Unclear
Wodehouse 2003	Low	Low	Unclear

Figure E2.3 – Forest plot for included articles for Question 2

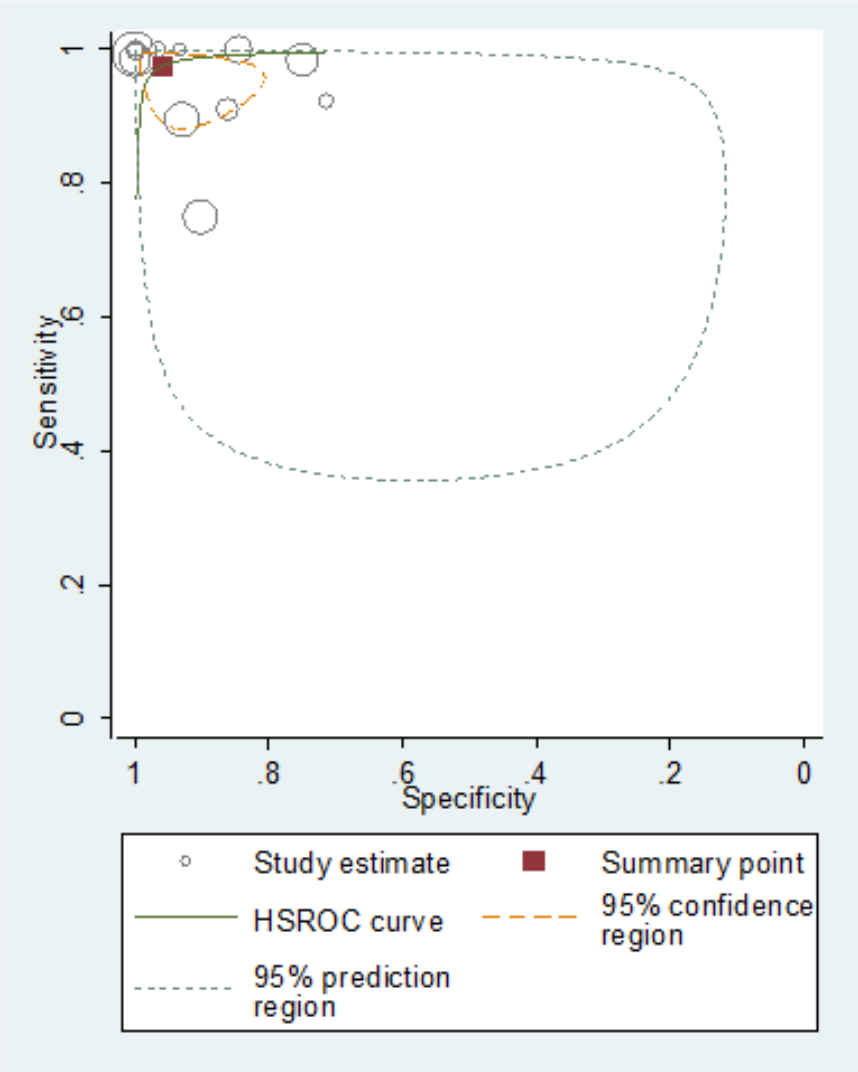
Study	TP	FP	FN	TN	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)
Santamaria 2008	13	1	0	14	17.0	1.00 [0.75, 1.00]	0.93 [0.68, 1.00]
Piacentini 2008	8	0	0	27	20.0	1.00 [0.63, 1.00]	1.00 [0.87, 1.00]
Harris 2014	11	1	0	29	38.0	1.00 [0.72, 1.00]	0.97 [0.83, 1.00]
Wodehouse 2003	30	12	0	66	50.0	1.00 [0.88, 1.00]	0.85 [0.75, 0.92]
Mateos Coral 2011	20	0	0	33	59.0	1.00 [0.83, 1.00]	1.00 [0.89, 1.00]
Leigh 2013 (lead site)	148	0	1	147	77.0	0.99 [0.96, 1.00]	1.00 [0.98, 1.00]
Leigh 2013 (other sites)	70	21	1	63	77.0	0.99 [0.92, 1.00]	0.75 [0.64, 0.84]
Beydon 2015	41	4	4	25	82.0	0.91 [0.79, 0.98]	0.86 [0.68, 0.96]
Boon 2014	34	10	4	132	90.0	0.89 [0.75, 0.97]	0.93 [0.87, 0.97]
Pifferi 2011	30	13	10	120	96.0	0.75 [0.59, 0.87]	0.90 [0.84, 0.95]
Noone 2004	68	0	1	71	100.0	0.99 [0.92, 1.00]	1.00 [0.95, 1.00]
Papon 2012	12	6	1	15	100.0	0.92 [0.64, 1.00]	0.71 [0.48, 0.89]



Forest plot (in ascending order of nasal nitric oxide cutoff value in nanoliters per minute). CI = confidence interval; FN = false negative; FP = false positive; TN = true negative; TP = true positive.

1. Santamaria F, De Stefano S, Montella S, Barbarano F, Iacotucci P, Ciccarelli R, Sofia M, Maniscalco M. Nasal nitric oxide assessment in primary ciliary dyskinesia using aspiration, exhalation, and humming. *Med Sci Monit* 2008; 14: CR80-85.
2. Piacentini GL, Bodini A, Peroni D, Rigotti E, Pigozzi R, Pradal U, Boner AL. Nasal nitric oxide for early diagnosis of primary ciliary dyskinesia: practical issues in children. *Respir Med* 2008; 102: 541-547.
3. Harris A, Bhullar E, Gove K, Joslin R, Pelling J, Evans HJ, Walker WT, Lucas JS. Validation of a portable nitric oxide analyzer for screening in primary ciliary dyskinesias. *BMC polm* 2014; 14: 18.
4. Wodehouse T, Kharitonov SA, Mackay IS, Barnes PJ, Wilson R, Cole PJ. Nasal nitric oxide measurements for the screening of primary ciliary dyskinesia. *Eur Respir J* 2003; 21: 43-47.
5. Mateos-Corral D, Coombs R, Grasemann H, Ratjen F, Dell SD. Diagnostic value of nasal nitric oxide measured with non-velum closure techniques for children with primary ciliary dyskinesia. *J Pediatr* 2011; 159: 420-424.
6. Leigh MW, Hazucha MJ, Chawla KK, Baker BR, Shapiro AJ, Brown DE, Lavange LM, Horton BJ, Qaqish B, Carson JL, Davis SD, Dell SD, Ferkol TW, Atkinson JJ, Olivier KN, Sagel SD, Rosenfeld M, Milla C, Lee HS, Krischer J, Zariwala MA, Knowles MR. Standardizing nasal nitric oxide measurement as a test for primary ciliary dyskinesia. *Ann Am Thorac Soc* 2013; 10: 574-581.
7. Beydon N, Chambellan A, Alberti C, de Blic J, Clément A, Escudier E, Le Bourgeois M. Technical and practical issues for tidal breathing measurements of nasal nitric oxide in children. *Pediatr Pulmonol* 2015.
8. Boon M, Meyts I, Proesmans M, Vermeulen FL, Jorissen M, De Boeck K. Diagnostic accuracy of nitric oxide measurements to detect primary ciliary dyskinesia. *Eur J Clin Invest* 2014; 44: 477-485.
9. Pifferi M, Bush A, Maggi F, Michelucci A, Ricci V, Conidi ME, Cangiotti AM, Bodini A, Simi P, Macchia P, Boner AL. Nasal nitric oxide and nitric oxide synthase expression in primary ciliary dyskinesia. *Eur Respir J* 2011; 37: 572-577.
10. Noone PG, Leigh MW, Sannuti A, Minnix SL, Carson JL, Hazucha M, Zariwala MA, Knowles MR. Primary ciliary dyskinesia: diagnostic and phenotypic features. *Am J Respir Crit Care Med* 2004; 169: 459-467.
11. Papon JF, Bassinet L, Cariou-Patron G, Zerah-Lancner F, Vojtek AM, Blanchon S, Crestani B, Amselem S, Coste A, Housset B, Escudier E, Louis B. Quantitative analysis of ciliary beating in primary ciliary dyskinesia: a pilot study. *Orphanet J Rare Dis* 2012; 7: 78.

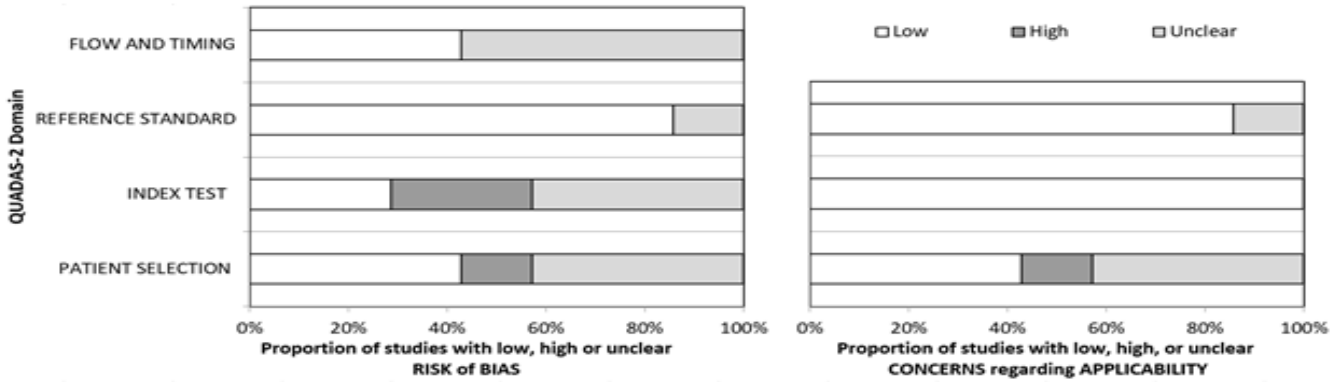
Figure E2.4 – HSROC for 12 included studies for Question 2



Hierarchical summary receiver operating characteristic curve (HSROC) for the 12 included studies.

1. Santamaria F, De Stefano S, Montella S, Barbarano F, Iacotucci P, Ciccarelli R, Sofia M, Maniscalco M. Nasal nitric oxide assessment in primary ciliary dyskinesia using aspiration, exhalation, and humming. *Med Sci Monit* 2008; 14: CR80-85.
2. Piacentini GL, Bodini A, Peroni D, Rigotti E, Pigozzi R, Pradal U, Boner AL. Nasal nitric oxide for early diagnosis of primary ciliary dyskinesia: practical issues in children. *Respir Med* 2008; 102: 541-547.
3. Harris A, Bhullar E, Gove K, Joslin R, Pelling J, Evans HJ, Walker WT, Lucas JS. Validation of a portable nitric oxide analyzer for screening in primary ciliary dyskinesias. *BMC polm* 2014; 14: 18.
4. Wodehouse T, Kharitonov SA, Mackay IS, Barnes PJ, Wilson R, Cole PJ. Nasal nitric oxide measurements for the screening of primary ciliary dyskinesia. *Eur Respir J* 2003; 21: 43-47.
5. Mateos-Corral D, Coombs R, Grasemann H, Ratjen F, Dell SD. Diagnostic value of nasal nitric oxide measured with non-velum closure techniques for children with primary ciliary dyskinesia. *J Pediatr* 2011; 159: 420-424.
6. Leigh MW, Hazucha MJ, Chawla KK, Baker BR, Shapiro AJ, Brown DE, Lavange LM, Horton BJ, Qaqish B, Carson JL, Davis SD, Dell SD, Ferkol TW, Atkinson JJ, Olivier KN, Sagel SD, Rosenfeld M, Milla C, Lee HS, Krischer J, Zariwala MA, Knowles MR. Standardizing nasal nitric oxide measurement as a test for primary ciliary dyskinesia. *Ann Am Thorac Soc* 2013; 10: 574-581.
7. Beydon N, Chambellan A, Alberti C, de Blic J, Clément A, Escudier E, Le Bourgeois M. Technical and practical issues for tidal breathing measurements of nasal nitric oxide in children. *Pediatr Pulmonol* 2015.
8. Boon M, Meyts I, Proesmans M, Vermeulen FL, Jorissen M, De Boeck K. Diagnostic accuracy of nitric oxide measurements to detect primary ciliary dyskinesia. *Eur J Clin Invest* 2014; 44: 477-485.
9. Pifferi M, Bush A, Maggi F, Michelucci A, Ricci V, Conidi ME, Cangiotti AM, Bodini A, Simi P, Macchia P, Boner AL. Nasal nitric oxide and nitric oxide synthase expression in primary ciliary dyskinesia. *Eur Respir J* 2011; 37: 572-577.
10. Noone PG, Leigh MW, Sannuti A, Minnix SL, Carson JL, Hazucha M, Zariwala MA, Knowles MR. Primary ciliary dyskinesia: diagnostic and phenotypic features. *Am J Respir Crit Care Med* 2004; 169: 459-467.
11. Papon JF, Bassinet L, Cariou-Patron G, Zerah-Lancner F, Vojtek AM, Blanchon S, Crestani B, Amselem S, Coste A, Housset B, Escudier E, Louis B. Quantitative analysis of ciliary beating in primary ciliary dyskinesia: a pilot study. *Orphanet J Rare Dis* 2012; 7: 78.

Figure E2.5 - Assessment of validity of individual studies with QUADAS for Question 2



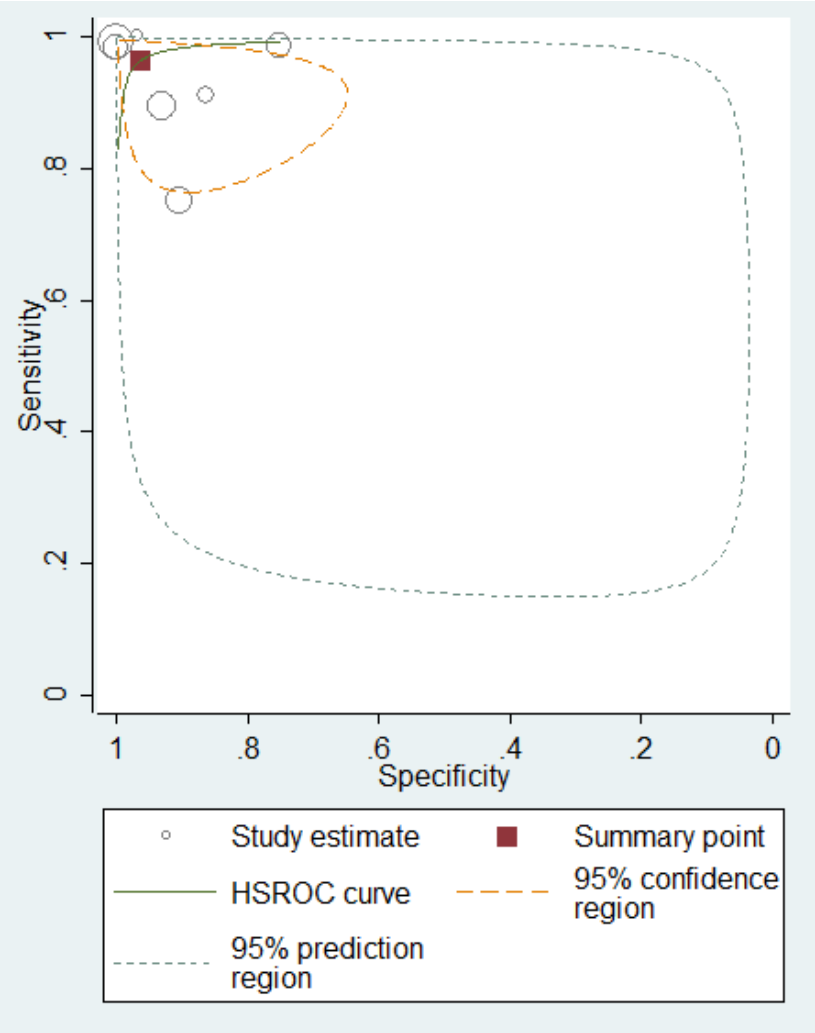
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Beydon 2015	Low	High	Unclear	Low
Boon 2014	Unclear	Low	Low	Unclear
Harris 2014	Unclear	High	Low	Unclear
Leigh 2013 (leading site)	Unclear	Unclear	Low	Unclear
Leigh 2013 (other sites)	Low	Low	Low	Low
Noone 2004	High	Unclear	Low	Unclear
Pfierri 2011	Low	Unclear	Low	Low

	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Beydon 2015	Low	Low	Low
Boon 2014	Unclear	Low	Low
Harris 2014	Unclear	Low	Low
Leigh 2013 (leading site)	Unclear	Low	Low
Leigh 2013 (other sites)	Low	Low	Low
Noone 2004	High	Low	Unclear
Pfierri 2011	Low	Low	Low

Assessment of validity of individual studies with Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 tool for the seven included studies comparing nasal nitric oxide to an extended reference standard of electron microscopy and/or genetics. The QUADAS-2 tool is designed to assess the quality of primary diagnostic accuracy studies and consists of four key domains evaluating the methods used with regard to patient selection, index test, reference standard, and flow of patients through the study, as well as timing of the index test and reference standard. The results presented show that the 7 selected studies were at lower risk of bias and concern regarding applicability than the initial 12 analyzed studies presented in Figure 2.2.

1. Beydon N, Chambellan A, Alberti C, de Blic J, Clément A, Escudier E, Le Bourgeois M. Technical and practical issues for tidal breathing measurements of nasal nitric oxide in children. *Pediatr Pulmonol* 2015.
2. Boon M, Meyts I, Proesmans M, Vermeulen FL, Jorissen M, De Boeck K. Diagnostic accuracy of nitric oxide measurements to detect primary ciliary dyskinesia. *Eur J Clin Invest* 2014; 44: 477-485.
3. Harris A, Bhullar E, Gove K, Joslin R, Pelling J, Evans HJ, Walker WT, Lucas JS. Validation of a portable nitric oxide analyzer for screening in primary ciliary dyskinesias. *BMC polm* 2014; 14: 18.
4. Leigh MW, Hazucha MJ, Chawla KK, Baker BR, Shapiro AJ, Brown DE, Lavange LM, Horton BJ, Qaqish B, Carson JL, Davis SD, Dell SD, Ferkol TW, Atkinson JJ, Olivier KN, Sagel SD, Rosenfeld M, Milla C, Lee HS, Krischer J, Zariwala MA, Knowles MR. Standardizing nasal nitric oxide measurement as a test for primary ciliary dyskinesia. *Ann Am Thorac Soc* 2013; 10: 574-581.
5. Noone PG, Leigh MW, Sannuti A, Minnix SL, Carson JL, Hazucha M, Zariwala MA, Knowles MR. Primary ciliary dyskinesia: diagnostic and phenotypic features. *Am J Respir Crit Care Med* 2004; 169: 459-467.
6. Pifferi M, Bush A, Maggi F, Michelucci A, Ricci V, Conidi ME, Cangiotti AM, Bodini A, Simi P, Macchia P, Boner AL. Nasal nitric oxide and nitric oxide synthase expression in primary ciliary dyskinesia. *Eur Respir J* 2011; 37: 572-577.

Figure E2.6 – HSROC for the 7 studies comparing nNO to an extended reference standard of electron microscopy and/or genetics



Hierarchical summary receiver operating characteristic curve (HSROC) for the seven studies comparing nasal nitric oxide to an extended reference standard of electron microscopy and/or genetics.

Table E2.1: Study and patient characteristics for Question 2

Study, year (reference)	Location	Study design	Patients, total n*	Patient description	PCD patients, n (prevalence)	Age	Gender, n male (%)
Beydon, 2015 (1)	France	Cohort	-86 patients suspected of having PCD	Patients included children with chronic rhino-sinusitis, serous otitis media, bronchiectasis, chronic bronchitis, or *situs inversus*	49 PCD total; Only 44 PCD performed nNO test correctly 49/86 (57.0%)	PCD median = 11.4 yo (range 7-13.9) Non-PCD median = 7.9 yo (range 4.9-11.6)	81/142 (57.0%)
Boon, 2014 (2)	Belgium	Case-control	191 patients: -38 PCD -153 non-PCD (51 HC, 48 asthma, 54 humoral immunodeficiency)	PCD patients included children and adults with recurrent upper or lower respiratory tract infections +/- organ *situs* anomalies	38 (NA)	Range = 5 to 25 yo PCD = 14.3 yo (range 8.8-18.1) Non-PCD = HC 14.9 yo (range 10.8-20.4), asthma 12.1 yo (range 9.8-16.5), humoral immunodeficiency = 10.7 yo (range 8.2-15.6)	85/191 (44.5%)
Harris, 2014 (3)	United Kingdom	Case-control	44 patients: -13 PCD -31 non-PCD (16 with symptoms, 15 HC)	Unclear	13 (NA)	Range = 6 to 79 yo	Not given

Leigh (leading site), 2013 (4)	United States	Case-control	296 patients : -149 PCD -147 non-PCD (37 asthma, 32 COPD and 78 HC)	PCD patients included children and adults with respiratory features suggestive of PCD (unexplained neonatal respiratory distress, year-round nasal congestion, year-round wet cough, >5 episodes of otitis media by 2 yo, or *situs* anomalies, usually after cystic fibrosis & immunodeficiency excluded	149 (NA)	PCD mean= 19.1 ± 14.8 yo Non-PCD mean = HC 20.9 ± 15.7 yo, asthma 14.8 ± 11.5 yo, COPD 61.1 ± 8.9 yo	139/296 (47.0%)
Leigh (other sites), 2013 (4)	United States	Cohort	155 patients suspected of having PCD	Patients included children and adults with respiratory features suggestive of PCD (unexplained neonatal respiratory distress, year-round nasal congestion, year-round wet cough, >5 episodes of otitis media by 2 yo, or *situs* anomalies, usually after cystic fibrosis & immunodeficiencies excluded	71/155 (45.8%)	PCD mean = 23.3 ± 18 yo Non-PCD mean = 31.8 ±22.3 yo	64/155 (41.3%)
Mateos Coral, 2011 (5)	Canada	Case-control	53 patients: -20 PCD -33 non-PCD (14 with bronchiectasis, 19 HC)	PCD patients included children with sinopulmonary symptoms typical of PCD, with CF and immunodeficiency ruled out	20 (NA)	PCD mean = 11.4 ± 3.5 yo Bronchiectasis mean = 10.9 ±3.3 yo, HC mean = 11.0 ± 3.7 yo	26/53 (49.1%)
Noone, 2014 (6)	United States	Case-control	140 patients: -69 PCD -71 non-PCD (27 HC, 44 healthy heterozygotes)	PCD patients included children and adults with lower airway disease with productive cough, wheeze, or shortness of breath and chronic upper airway	69 (NA)	PCD children median = 8 yo (range 1-17) PCD adults median = 36	PCD: 36/78 (46.2%)

				symptoms of rhinitis/sinusitis +/- *situs* inversus totalis.		yo (range 19-73) Non-PCD means = HC 37 ± 2 yo, and healthy heterozygotes = 44 ± 2 yo	
Papon, 2012 (7)	France	Cohort	34 patients suspected of having PCD	Patients included children and adults with chronic upper and/or lower respiratory tract infections, bronchitis, bronchiectasis, and sinusitis.	13/34 (38.2%)	Mean = 32.5 yo (range 10-72)	16/34 (47.1%)
Piacentini, 2008 (8)	Italy	Case-control	-35 patients: -8 PCD -27 non-PCD (HC)	PCD patients included children with *situs inversus* and/or bronchiectasis and/or sinusitis	10 PCD total; Only 8 performed nNO test correctly (NA)	PCD mean = 17 yo; Non-PCD = 27 school aged with mean of 7 yo	53/87 (60.9%)
Pifferi, 2011 (9)	Italy	Cohort	-173 patients suspected of having PCD	Patients included children with clinical history and symptoms of PCD, without cystic fibrosis, aspiration, gastro-esophageal reflux, or immunodeficiency.	48 PCD total; Only 40 PCD performed nNO test correctly 48/173 (27.7%)	Median = 6.2 yo (range 1 mo to 17.5)	105/209 (50.2%)
Santamaria, 2008 (10)	Italy	Case-control	28 patients -14 PCD -14 non-PCD (14 HC)	Unclear	14 (NA)	PCD mean = 15 yo (range = 7-27) HC mean = 16 yo (range = 7-27)	18/28 (64.3%)
Wodehouse, 2003 (11)	United Kingdom	Case-control	108 patients: -42 PCD -66 non-PCD (20 with bronchiectasis,	Unclear	42 (NA)	PCD mean = 34.2 ± 10.9 yo Non-PCD range of	48/108 (44.4%)

			12 Young's syndrome, 18 sinusitis, 16 HC)			means = 36.2 to 53.2 yo	

*Number of patients included in our final analysis after excluding patients experiencing technical difficulties with nNO testing (Beydon (n=39) and Pifferi (n=3)), CF subjects (Boon (n=50), Harris (n=6), Leigh (lead site) (n=77), Mateos Coral (n=32), Noone (n=11), and Wodehouse (n=15)), and patients with an inconclusive reference standard result (Beydon (n=56)). Additionally, uncooperative children who could only perform tidal breathing nNO measurements were excluded from analysis (Beydon (PCD n=5, non-PCD n=7), Piacentini (PCD n=2, Healthy controls n=50), and Pifferi (PCD n=8, non-PCD=28)).

CF – cystic fibrosis, HC – healthy control, NA – not applicable, nNO – nasal nitric oxide, yo – years old

1. Beydon N, Chambellan A, Alberti C, de Blic J, Clément A, Escudier E, Le Bourgeois M. Technical and practical issues for tidal breathing measurements of nasal nitric oxide in children. *Pediatr Pulmonol* 2015.
2. Boon M, Meyts I, Proesmans M, Vermeulen FL, Jorissen M, De Boeck K. Diagnostic accuracy of nitric oxide measurements to detect primary ciliary dyskinesia. *Eur J Clin Invest* 2014; 44: 477-485.
3. Harris A, Bhullar E, Gove K, Joslin R, Pelling J, Evans HJ, Walker WT, Lucas JS. Validation of a portable nitric oxide analyzer for screening in primary ciliary dyskinesias. *BMC polm* 2014; 14: 18.
4. Leigh MW, Hazucha MJ, Chawla KK, Baker BR, Shapiro AJ, Brown DE, Lavange LM, Horton BJ, Qaqish B, Carson JL, Davis SD, Dell SD, Ferkol TW, Atkinson JJ, Olivier KN, Sagel SD, Rosenfeld M, Milla C, Lee HS, Krischer J, Zariwala MA, Knowles MR. Standardizing nasal nitric oxide measurement as a test for primary ciliary dyskinesia. *Ann Am Thorac Soc* 2013; 10: 574-581.
5. Mateos-Corral D, Coombs R, Grasemann H, Ratjen F, Dell SD. Diagnostic value of nasal nitric oxide measured with non-velum closure techniques for children with primary ciliary dyskinesia. *J Pediatr* 2011; 159: 420-424.
6. Noone PG, Leigh MW, Sannuti A, Minnix SL, Carson JL, Hazucha M, Zariwala MA, Knowles MR. Primary ciliary dyskinesia: diagnostic and phenotypic features. *Am J Respir Crit Care Med* 2004; 169: 459-467.
7. Papon JF, Bassinet L, Cariou-Patron G, Zerah-Lancner F, Vojtek AM, Blanchon S, Crestani B, Amselem S, Coste A, Housset B, Escudier E, Louis B. Quantitative analysis of ciliary beating in primary ciliary dyskinesia: a pilot study. *Orphanet J Rare Dis* 2012; 7: 78.
8. Piacentini GL, Bodini A, Peroni D, Rigotti E, Pigozzi R, Pradal U, Boner AL. Nasal nitric oxide for early diagnosis of primary ciliary dyskinesia: practical issues in children. *Respir Med* 2008; 102: 541-547.
9. Pifferi M, Bush A, Maggi F, Michelucci A, Ricci V, Conidi ME, Cangiotti AM, Bodini A, Simi P, Macchia P, Boner AL. Nasal nitric oxide and nitric oxide synthase expression in primary ciliary dyskinesia. *Eur Respir J* 2011; 37: 572-577.
10. Santamaria F, De Stefano S, Montella S, Barbarano F, Iacotucci P, Ciccarelli R, Sofia M, Maniscalco M. Nasal nitric oxide assessment in primary ciliary dyskinesia using aspiration, exhalation, and humming. *Med Sci Monit* 2008; 14: CR80-85.
11. Wodehouse T, Kharitonov SA, Mackay IS, Barnes PJ, Wilson R, Cole PJ. Nasal nitric oxide measurements for the screening of primary ciliary dyskinesia. *Eur Respir J* 2003; 21: 43-47.

Table E2.2: Index test and reference standard characteristics for Question 2

Study, year (reference)	Index test characteristics*				Reference standard characteristics*			
	Analyser	Flow rate (L/min)	Method	Cut-off (nL/min)	PCD diagnosis	TEM ultrastructure	Genetic	PCD diagnosis not confirmed by TEM and/or genetics
Beydon, 2015** (1)	NIOX Flex, Endono 8000	0.30	Mainly ER, 5 PCD via TB were excluded	82.2	44 of 49 PCD analysed: TEM (n=44) and/or genetics (n=22)	ODA (n=17) ODA+IDA (n=5) Central pair (n=10)	DNAI1 (n=5) DNAI2 (n=1) RSPH1 (n=1) RSPH9 (n=1) RSPH4A (n=2) DYX1C1 (n=2) RPGR (n=1) *-Unknown total number of genes tested*	3 IDA defects alone without confirmation by genetics (6.8%)
						IDA+MTD (n=9)	CCDC39 (n=6) CCDC40 (n=3) *-Unknown total number of genes tested*	
						IDA alone (n=3)		
Boon, 2014** (2)	EcoPhysics CLD88	0.30	ER	90	38 PCD analysed: TEM (n=23) or HSVM after ciliary culture regrowth (n=15), *and/or post hoc confirmation by*	*ODA (n=19)*	*DNAH5 (n=4)* *-Only DNAH5 tested*	2 normal TEM without confirmation by genetics (5.1%)
						IDA+MTD (n=3)	*CCDC40 (n=3)* *-Only CCDC40 tested*	
						RSP (n=1)	*RSPH4 (n=1)* *-Unknown total number of genes tested*	
						Normal TEM with	*DNAH11 (n=10)*	

					genetics (n=21)	*abnormal HSVM (n=15)*	*-Exome sequence used for 10 cases* *HYDIN (n=2)* *CCDC65 (n=1)* *-Unknown total number of genes tested*	
Harris, 2014** (3)	NIOX Flex	0.30	BH	38	13 PCD analysed: TEM (n=11) or HSVM after ciliary culture regrowth in some cases *with post hoc confirmation by genetics (n=2)*	*ODA (n=5)* *ODA+IDA (n=5)* *IDA+MTD (n=1)*		0
						Normal TEM with abnormal HSVM (n=2)	*DNAH11 (n=2)* *-Only DNAH11 tested*	
Leigh (leading site), 2013** (4)	Sievers 280i, EcoPhysics CLD88, NIOX Flex	0.50, 0.33, 0.30	ER	76.9	149 PCD analysed: TEM (n=143) or genetics (n=6)	*ODA (n=87)* *ODA+IDA (n=28)* *IDA+MTD (n=23)* *CA (n=5)*		0
						Normal TEM (n=6)	DNAH11 (n=6)	
Leigh (other sites), 2013** (4)	Sievers 280i, EcoPhysics CLD88, NIOX Flex	0.50, 0.33, 0.30	ER	76.9	71 PCD analysed: TEM (n=65) or genetics (n=6)	*ODA (n=36)* *ODA+IDA (n=13)* *IDA+MTD (n=15)* *CA (n=1)*		0

						Normal TEM (n=3) *Inadequate TEM (n=3)*	*Confirmed but not disclosed (n=6)* *-Unknown total number of genes tested*	
Mateos Coral, 2011 (5)	EcoPhysics CLD88	0.33	ER	58.5	20 PCD analysed: TEM (n=20) *with post hoc confirmation by genetics (n=17)*	ODA+IDA (n=11) IDA+MTD (n=4) ODA (n=3) RSP (n=2)	*DNAH5 (n=6)* *DNAH11 (n=1)* *DNAI2 (n=1)* *CCDC39 (n=2)* *CCDC40 (n=1)* *DYX1C1 (n=3)* *RSHP4A (n=1)* *KTU (n=1)* *LRRC50 (n=1)* *-2 gene panel used in 1 case* *-12 gene panel used in 12 cases* *-21 gene panel used in 3 cases* *-32 gene panel used in 4 cases*	0
Noone, 2014**(6) (7)	Sievers 270B	0.50	BH	*100*	69 PCD analysed: TEM (n=60) or complete clinical phenotype *with post hoc confirmation by genetics (n=9)*	ODA (n=31) ODA+IDA (n=16) *IDA+MTD (n=13)*	*Confirmed but not disclosed (n=9)* *-Only 2 genes tested*	0

Papon, 2012 (8)	EVA4000	per ATS standards	per ATS standards	100	13 PCD analysed: TEM (n=13)	ODA (n=9) IDA+nexin link (n=2) ODA+IDA (n=1) Central pair (n=1)		0
Piacentini, 2008 (9)	NIOX Flex	0.30	Mainly BH, 2 PCD via TB were excluded	20.4	8 of 10 PCD analysed: TEM (n=10)	ODA+IDA (n=7) ODA (n=1) IDA (n=2)		0
Pifferi, 2011** (10)	*EcoPhysics CLD88*	*0.33*	*Mainly ER, 8 PCD via TB were excluded*	96	40 of 48 PCD analysed: TEM (n=42) or HSVM after ciliary culture regrowth *with post hoc confirmation by genetics (n=6)*	*ODA+IDA (n=23)* *IDA+CA+MTD (n=12)* *ODA (n=2)* *IDA+MTD (n=3)* *IDA (n=2)*		0
						Normal TEM with abnormal HSVM (n=6)	*DNAH11 (n=6)* *-Only DNAH11 tested*	
Santamaria, 2008 (11)	NIOX Flex	0.28	BH	16.8	14 PCD analysed: TEM (n=14)	ODA+IDA (n=8) ODA (n=1) IDA+MTD (n=3) Central pair (n=1)		1 non-classic TEM anomaly without confirmation by genetics (7.1%)
						Basal body anomaly (n=1)		
Wodehouse, 2003 (6)	LR2000	0.25	BH	50	42 PCD analysed: TEM (n=42)	ODA (n=21) ODA+IDA (n=5)		12 IDA defects alone without confirmation by

						Transposition (n=2) Radial spoke (n=2)		genetics (28.6%)
						Unspecified IDA (n=12)		

ATS – American Thoracic Society; BH - breath hold; CA - Central apparatus defect; ER - exhalation against resistance; HSVM – high speed videomicroscopy; IDA - Inner dynein arm; IDA+MTD - Inner dynein arm + microtubule disorganization defect; ODA - Outer dynein arm defect; ODA+IDA - Outer dynein arm + Inner dynein arm defect; PCD – Primary Ciliary Dyskinesia; TB - tidal breathing; TEM - transmission electron microscopy;

*All information in *italics* are from personal communication with the authors

**Studies considered as using a combination of TEM and/or genetics as the reference standard

1. Beydon N, Chambellan A, Alberti C, de Blic J, Clément A, Escudier E, Le Bourgeois M. Technical and practical issues for tidal breathing measurements of nasal nitric oxide in children. *Pediatr Pulmonol* 2015.
2. Boon M, Meyts I, Proesmans M, Vermeulen FL, Jorissen M, De Boeck K. Diagnostic accuracy of nitric oxide measurements to detect primary ciliary dyskinesia. *Eur J Clin Invest* 2014; 44: 477-485.
3. Harris A, Bhullar E, Gove K, Joslin R, Pelling J, Evans HJ, Walker WT, Lucas JS. Validation of a portable nitric oxide analyzer for screening in primary ciliary dyskinesias. *BMC polm* 2014; 14: 18.
4. Leigh MW, Hazucha MJ, Chawla KK, Baker BR, Shapiro AJ, Brown DE, Lavange LM, Horton BJ, Qaqish B, Carson JL, Davis SD, Dell SD, Ferkol TW, Atkinson JJ, Olivier KN, Sagel SD, Rosenfeld M, Milla C, Lee HS, Krischer J, Zariwala MA, Knowles MR. Standardizing nasal nitric oxide measurement as a test for primary ciliary dyskinesia. *Ann Am Thorac Soc* 2013; 10: 574-581.
5. Mateos-Corral D, Coombs R, Grasemann H, Ratjen F, Dell SD. Diagnostic value of nasal nitric oxide measured with non-velum closure techniques for children with primary ciliary dyskinesia. *J Pediatr* 2011; 159: 420-424.
6. Wodehouse T, Kharitonov SA, Mackay IS, Barnes PJ, Wilson R, Cole PJ. Nasal nitric oxide measurements for the screening of primary ciliary dyskinesia. *Eur Respir J* 2003; 21: 43-47.
7. Noone PG, Leigh MW, Sannuti A, Minnix SL, Carson JL, Hazucha M, Zariwala MA, Knowles MR. Primary ciliary dyskinesia: diagnostic and phenotypic features. *Am J Respir Crit Care Med* 2004; 169: 459-467.
8. Papon JF, Bassinet L, Cariou-Patron G, Zerah-Lancner F, Vojtek AM, Blanchon S, Crestani B, Amselem S, Coste A, Housset B, Escudier E, Louis B. Quantitative analysis of ciliary beating in primary ciliary dyskinesia: a pilot study. *Orphanet J Rare Dis* 2012; 7: 78.
9. Piacentini GL, Bodini A, Peroni D, Rigotti E, Pigozzi R, Pradal U, Boner AL. Nasal nitric oxide for early diagnosis of primary ciliary dyskinesia: practical issues in children. *Respir Med* 2008; 102: 541-547.
10. Pifferi M, Bush A, Maggi F, Michelucci A, Ricci V, Conidi ME, Cangiotti AM, Bodini A, Simi P, Macchia P, Boner AL. Nasal nitric oxide and nitric oxide synthase expression in primary ciliary dyskinesia. *Eur Respir J* 2011; 37: 572-577.
11. Santamaria F, De Stefano S, Montella S, Barbarano F, Iacotucci P, Ciccarelli R, Sofia M, Maniscalco M. Nasal nitric oxide assessment in primary ciliary dyskinesia using aspiration, exhalation, and humming. *Med Sci Monit* 2008; 14: CR80-85.

Table E2.3: Summary of findings table including the 7 studies comparing nNO to an extended reference standard of TEM and/ or genetics for Question 2

Sensitivity	0.96 (95% CI: 0.89 to 0.99)
Specificity	0.96 (95% CI: 0.85 to 0.99)

Prevalence	35% (1)

Outcome	№ of studies (№ of patients)	Study design	Factors that may decrease quality of evidence					Effect per 100 patients tested	Test accuracy QoE	Importance
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 35%		
True positives (patients with PCD)	7 studies 423 patients	cohort & case-control type studies	serious[a]	not serious	not serious	not serious	None	34 (31 to 35)	⊕⊕⊕◯ MODERATE	CRITICAL
False negatives (patients incorrectly classified as not having PCD)								1 (0 to 4)		CRITICAL
True negatives (patients without PCD)	7 studies 636 patients	cohort & case-control type studies	serious[a]	not serious	not serious	not serious	None	63 (55 to 64)	⊕⊕⊕◯ MODERATE	CRITICAL
False positives (patients incorrectly classified as having PCD)								2 (1 to 10)		IMPORTANT
Inconclusive	7 studies 27 patients	-	-	-	-	-	-		-	IMPORTANT

Definition of abbreviations: CI = confidence interval; PCD = primary ciliary dyskinesia; QOE = quality of evidence.
*Sensitivity, 0.96 (95% confidence interval, 0.89–0.99); specificity, 0.96 (95% confidence interval, 0.85–0.99); prevalence 35%.
†Four studies were case–control studies, among which one study included only healthy patients in the control group. Two studies did not prespecify the nasal nitric oxide cutoff before performing measurements and were not blinded to the reference standard.
‡Not downgraded for inconsistency since the residual heterogeneity was explained by the difference in the risk of bias between studies

1. Leigh MW, Ferkol TW, Davis SD, Lee HS, Rosenfeld M, Dell SD, Sagel SD, Milla C, Olivier KN, Sullivan KM, Zariwala MA, Pittman JE, Shapiro AJ, Carson JL, Krischer J, Hazucha MJ, Knowles MR. Clinical Features and Associated Likelihood of Primary Ciliary Dyskinesia in Children and Adolescents. *Ann Am Thorac Soc* 2016; 13: 1305-1313.
2. Beydon N, Chambellan A, Alberti C, de Blic J, Clément A, Escudier E, Le Bourgeois M. Technical and practical issues for tidal breathing measurements of nasal nitric oxide in children. *Pediatr Pulmonol* 2015.
3. Boon M, Meyts I, Proesmans M, Vermeulen FL, Jorissen M, De Boeck K. Diagnostic accuracy of nitric oxide measurements to detect primary ciliary dyskinesia. *Eur J Clin Invest* 2014; 44: 477-485.
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5. Leigh MW, Hazucha MJ, Chawla KK, Baker BR, Shapiro AJ, Brown DE, Lavange LM, Horton BJ, Qaqish B, Carson JL, Davis SD, Dell SD, Ferkol TW, Atkinson JJ, Olivier KN, Sagel SD, Rosenfeld M, Milla C, Lee HS, Krischer J, Zariwala MA, Knowles MR. Standardizing nasal nitric oxide measurement as a test for primary ciliary dyskinesia. *Ann Am Thorac Soc* 2013; 10: 574-581.
6. Noone PG, Leigh MW, Sannuti A, Minnix SL, Carson JL, Hazucha M, Zariwala MA, Knowles MR. Primary ciliary dyskinesia: diagnostic and phenotypic features. *Am J Respir Crit Care Med* 2004; 169: 459-467.
7. Pifferi M, Bush A, Maggi F, Michelucci A, Ricci V, Conidi ME, Cangiotti AM, Bodini A, Simi P, Macchia P, Boner AL. Nasal nitric oxide and nitric oxide synthase expression in primary ciliary dyskinesia. *Eur Respir J* 2011; 37: 572-577.

Table E2.4 Evidence to Decision Table - Question 2

Should a low nasal nitric oxide level (with chemiluminescence technology), after ruling out cystic fibrosis, be used as a diagnostic test for PCD, in adult and pediatric patients >5 years old, who are at high probability of having PCD? *(as replacement of reference standards of classic TEM structural ciliary defect or biallelic causative mutations in PCD genes)?*

POPULATION:	Patients with a high pre-test probability	**BACKGROUND:** A growing number of clinical centers across North America employ nasal nitric oxide (nNO) measurements using a velum closure maneuver in patients (when cystic fibrosis has been ruled out) as a rapid and inexpensive screening test for PCD, before deciding to proceed to more labor and cost-intensive for definitive PCD diagnosis (TEM and/or genetics)(1, 2). A low nNO measurement (<77 nL/min) via a chemiluminescence analyzer is highly sensitive and specific for PCD diagnosed through classic TEM ciliary defects or biallelic mutations in a known PCD-causing gene (3). However, this test has only been validated in cooperative children (generally >5 years old), and current chemiluminescent devices are not clinically approved by regulatory agencies in North America. Furthermore, nNO values can be influenced by acute viral infections, acute sinusitis, and other rare diseases, requiring clinicians to ensure these conditions are not present or influencing nNO results. Nasal Nitric oxide testing for PCD is rapid, non-invasive, inexpensive from a consumables standpoint, and results are immediately available. Potential PCD patients with low nNO values will normally progress to other confirmatory testing with TEM ciliary analysis or genetic testing. Some individuals with low nNO values will have normal TEM testing and no causative mutations on genetic testing, yet they will still be treated for PCD if physicians cannot find alternative diagnoses to explain their chronic oto-sino-pulmonary symptoms.
INTERVENTION:	nNO measurements	
PURPOSE OF THE TEST:	Diagnosis of PCD	
LINKED TREATMENTS:	Targeted pulmonary/ENT care in a PCD specialized center in patients with confirmed PCD or further investigations for other potentially treatable diseases in patients with negative testing for PCD	
ANTICIPATED OUTCOMES:	Premature death, need for lung transplant, rapid deterioration of pulmonary function, restriction in physical functioning/activity, development of bronchiectasis, deterioration of overall quality of life, recurrent sinopulmonary exacerbations, recurrent hospitalizations, hearing loss or speech delay, recurrent antibiotics use, need for ear tube placement, need for sinus surgery, infertility, depression/anxiety and side effects of repeat testing, absenteeism, poor social functioning, resources use	
SETTING:	Outpatient setting	
PERSPECTIVE:	Clinical recommendation from an individual perspective	

Assessment – Question 2

	JUDGMENT	RESEARCH EVIDENCE	ADDITIONAL CONSIDERATIONS
PROBLEM	**Is the problem a priority?** ○ No ○ Probably no ○ Probably yes ○ **Yes** ○ Varies ○ Don't know	A growing number of clinical centers across North America employ nasal nitric oxide measurements using a velum closure maneuver in patients, where cystic fibrosis has been ruled out, as a rapid and inexpensive screening test for PCD before deciding to proceed to more labor and cost-intensive for definitive PCD diagnosis (TEM and/or genetics) (1, 2). However, both TEM ciliary analysis and PCD genetic testing are imperfect reference standards for PCD. TEM testing is difficult to perform correctly outside of highly experienced centers, with some major academic centers suffering from poor feasibility for this complex test (20-40% of samples are inconclusive or lack sufficient material for analysis) (4-6). In experienced research centers, this feasibility is greatly improved (7). Other centers misinterpret secondary ciliary changes on TEM as primary, disease-causing defects, leading to false positive results (8, 9). TEM analysis is also costly (approximately $1000 USD per test), and at least 10-20% of patients require repeat TEM testing to confirm their defects (6, 10). Some centers prefer lower airway samples for their TEM analysis (as opposed to nasal biopsies), requiring a general anesthesia in most pediatric patients (6). Lastly, TEM will be normal in approximately 30% of PCD cases confirmed by other testing (genetics, HSVM, immunofluorescent staining)(5, 11). Genetic testing also has limitations in PCD diagnosis, as the number of PCD-causing genes continues to grow rapidly and a complete panel does not exist (12). Currently, PCD-causing genetic mutations are only known in approximately 70% of all proven PCD cases (11, 12). Commercial PCD genetic testing is widely available in North America, but payment for this testing can be difficult to obtain through insurance and governmental coverages. The access to genetic testing in Europe is even more limited due to similar payment issues. While this test is highly feasible (requiring only peripheral venipuncture) and does not require physical access to specialized centers, results can be uninformative with frequently encountered variants of unknown significance (13-18).	

<table>
<tr>
<td>TEST ACCURACY</td>
<td>How accurate is the test?
○ Very inaccurate
○ Inaccurate
○ Accurate
○ Very accurate

○ Varies
○ Don't know</td>
<td>Using an extended reference standard of TEM and/or genetic testing, sensitivity of nNO measurements was 96.3% (95% CI 88.7-98.9) and specificity was 96.4% (85.1-99.2) in a population with a pre-test probability of 35%.
<table>
<tr><th>Test results</th><th>Importance</th><th>Effects per 100 patients tested (prevalence = 35%)</th><th>Quality of evidence</th></tr>
<tr><td>TP</td><td>Critical</td><td>34 (31 to 35)</td><td rowspan="2">⊕⊕⊕◯
MODERATE</td></tr>
<tr><td>FN</td><td>Critical</td><td>1 (0 to 4)</td></tr>
<tr><td>TN</td><td>Critical</td><td>63 (55 to 64)</td><td rowspan="2">⊕⊕⊕◯
MODERATE</td></tr>
<tr><td>FP</td><td>Important</td><td>2 (1 to 10)</td></tr>
</table>
</td>
<td>The panel considered that the excellent diagnostic accuracy of nNO may even be higher, as the reference standards of TEM and/or genetics are likely incorrect in some cases (based on the clinical phenotype (situs inversus, daily year-round wet cough and nasal congestion since infancy, and neonatal respiratory distress) and all other similar diseases (CF, immunodeficiency, etc.) have been ruled out). As more PCD-causing gene mutations are discovered, the diagnostic accuracy of nNO will likely improve even further.</td>
</tr>
<tr>
<td>DESIRABLE EFFECTS</td>
<td>How substantial are the desirable anticipated effects?
○ Trivial
○ Small
○ Moderate
○ Large

○ Varies
○ Don't know</td>
<td>
<table>
<tr><th></th><th>Index test +
(low nNO measurements)</th><th>Index test –
(high nNO measurements)</th></tr>
<tr><td>PCD +</td><td><u>TRUE POSITIVES</u>
-Referral to a PCD specialized center
-Rapid cessation of repeat testing, thus avoid unnecessary supplementary costs and anxiety over awaiting confirmation of PCD diagnosis
-PCD targeted pulmonary and ENT therapies with</td><td><u>FALSE NEGATIVES **</u>
-Discharge from a PCD specialized center (diagnosis of PCD will likely be <u>missed</u>)
-Unnecessary investigation for other diseases
-Unnecessary supplementary costs and anxiety over awaiting diagnosis
-No PCD targeted pulmonary and ENT care, and may receive other non-PCD cares with risks (e.g.</td></tr>
</table>
</td>
<td>*The panel considered that the undesirable downstream consequences of false positive results are difficult to assess and thus uncertain for 2 main reasons: 1) false positive results could still be PCD since ongoing studies show the references standards of TEM and genetic testing lack sensitivity to detect PCD (i.e. new genetic variants are discovered each year) 2) Increased heterogeneity in the non-PCD, true underlying disease, which will partially benefit from the expected effects of the PCD targeted pulmonary and ENT therapies, regardless of the diagnosis.

**The panel considered that the undesirable downstream consequences of false negative results difficult to assess and thus uncertain for</td>
</tr>
</table>

<table>
<tr>
<td>UNDESIRABLE EFFECTS</td>
<td>How substantial are the undesirable anticipated effects?
○ Large
○ Moderate
○ Small
○ Trivial

○ Varies
○ Don't know</td>
<td>
<table>
<tr><td></td><td>probable clinical improvement</td><td>IVIG with blood product exposures, lobectomy)</td></tr>
<tr><td>PCD -</td><td><u>FALSE POSITIVES *</u>
-Referral to a PCD specialized center (<u>diagnosis of the true disease will likely be delayed</u>)
-PCD targeted pulmonary and ENT care with possible clinical improvement regardless of the cause of chronic lung disease.
-No specific therapy for the true underlying disease, if it exists (e.g. IVIG for immunodeficiency)</td><td><u>TRUE NEGATIVES</u>
-Discharge from a PCD specialized center
-Investigation for other potentially treatable diseases (such as immunodeficiency)
-Rapid cessation of repeat testing, thus avoid unnecessary supplementary costs and anxiety over awaiting information of PCD diagnosis</td></tr>
</table>
</td>
<td>2 main reasons: 1) the effect could be have been underestimated since the studies assessing the impact of delayed diagnosis were not recently performed, and the standard of care has greatly improved (as well as the patient outcomes), 2) the effect could have been overestimated since older age at PCD diagnosis (usually correlated with delayed diagnosis) is associated with distrust in medical community, with less improvement in the St George's Respiratory Questionnaire scores, worsened long-term compliance with PCD treatment regimens (19) and ultimately, with worse outcomes (increased rates of respiratory cultures positive for Pseudomonas aeruginosa infection (20), which causes worse outcomes in similar respiratory diseases (21), increased rates of medical and surgical complications, including nasal polyposis, hemoptysis, and lobectomy surgery, all of which can cause significant morbidity and even mortality (22)).</td>
</tr>
<tr>
<td>CERTAINTY OF THE EVIDENCE OF TEST</td>
<td>What is the overall certainty of the evidence of test accuracy?
○ Very low
○ Low
○ Moderate
○ High</td>
<td>Risk of bias of included studies led to rating down the certainty in the evidence. Detailed judgment in provided in the evidence tables.</td>
<td></td>
</tr>
</table>

	○ No included studies		
CERTAINTY OF THE EVIDENCE OF TEST'S EFFECTS	**What is the overall certainty of the evidence for any critical or important direct benefits, adverse effects or burden of the test?** ○ Very low ○ Low ○ Moderate ○ High **○ No included studies**	No direct evidence for critical or important direct benefits, adverse effects or burden of the test (i.e. side effects of repeat testing and anxiety related to delayed diagnosis) was considered here.	The panel assumed that: 1) Nasal nitric oxide measurements require patients to travel to experienced centers, produce immediate results, give non-diagnostic results less often than TEM or genetic testing, and are painless without reported complications. 2) TEM analysis sometimes requires patients to travel to experienced centers, can take weeks to produce results, frequently gives non-diagnostic results requiring repeat biopsies (reported inconclusive results rates vary between 20% and 42% in experienced centers performing sampling under optimal conditions (4-6, 10, 23)), and complications of biopsy are minimal (mild discomfort, possibly mild bleeding)(24, 25). 3) Genetic testing does not require patient travel, but can take weeks to complete analysis, and can produce non-diagnostic results with variants of unknown significance (12) requiring other PCD diagnostic tests. Complications of venipuncture are minimal (mild discomfort).

			Thus, if TEM and/or genetics are replaced by nasal NO measurements, the panel concluded that patients will need to travel to specialized centers but results can be immediately available and less often indeterminate, resulting in earlier time to diagnosis and a lower proportion of indeterminate diagnoses. This strategy should ultimately reduce unnecessary side effects associated with repeat testing and anxiety related to delayed diagnosis.
CERTAINTY OF THE EVIDENCE OF MANAGEMENT'S EFFECTS	**What is the overall certainty of the evidence of effects of the management that is guided by the test results?** ○ Very low ○ **Low** ○ Moderate ○ High ○ No included studies	No direct evidence comparing PCD targeted pulmonary and ENT care versus no treatment was considered, since these treatments consist of a bundle of different supportive therapies which are usually (at least) partially started for symptom relief. Nevertheless, longitudinal PCD studies show that patients using long term standard PCD treatment regimens experienced less decline in lung function than patients left undiagnosed and thus untreated (26-28). Referral of pediatric patients to a PCD center of excellence for long-term therapies may also improve lung function and nutrition (29). Furthermore, later diagnosis (in adulthood) of PCD might be linked to worsened long-term pulmonary outcomes (26). Other individual interventions were occasionally studied but could not be pooled due to the heterogeneity of interventions and/or comparators for each critical outcome. For instance, children with PCD and chronic otitis media with effusion show marked improvements in hearing after surgical placement of ventilation tubes versus medical therapy alone (30, 31). Aggressive surgical management of chronic rhinosinusitis in PCD patients also provides significant symptom relief (32). Regular airway clearance also shows improvements in lung function in one small cross-over RCT (33).	The panel considered that standard PCD therapies are likely more efficient than what is currently reported, but equipoise would preclude studying the natural evolution of the disease without minimal intervention.

<table>
<tr>
<td>CERTAINTY OF THE EVIDENCE OF TEST RESULT/MANAGEMENT</td>
<td>How certain is the link between test results and management decisions?
○ Very low
○ Low
○ Moderate
○ High

○ No included studies</td>
<td>Observational studies showed that PCD patients will promptly begin standard therapies for PCD, including daily airway clearance, sputum culture surveillance, otolaryngology care, and aggressive use of antibiotics for respiratory infections (1, 34). Nevertheless, these therapies may be suboptimal outside of PCD specialized centers. Furthermore, erratic long-term compliance with PCD treatment regimens, especially in older patients at diagnosis (19), increases uncertainty regarding the link between testing and treatment.</td>
<td>The panel confirms that in clinical practice a positive diagnostic for PCD will almost certainly lead to the start of chronic therapies if patient is referred to a PCD specialized center.</td>
</tr>
<tr>
<td>CERTAINTY OF EFFECTS</td>
<td>What is the overall certainty of the evidence of effects of the test?
○ Very low
○ Low
○ Moderate
○ High

○ No included studies</td>
<td>The overall certainty of the evidence of the effects of testing and subsequent management decisions on patient-important outcomes is limited by the very low certainty regarding the link between tests results and management decisions and the low certainty of the effects of the management guided by the test results.</td>
<td></td>
</tr>
<tr>
<td>VALUES</td>
<td>Is there important uncertainty about or variability in how much people value the main outcomes?
○ Important uncertainty or variability
○ Possibly important uncertainty or</td>
<td>There are also numerous publications addressing the stress created in patients surrounding their difficulty obtaining a proper PCD diagnosis. Indeed, uncertainty surrounding PCD diagnosis has been linked to poor psychosocial outcomes (35, 36). Several PCD patients and family representatives of PCD patients sat on this committee, and they repeatedly voiced their frustration with poor quality diagnostic testing and ambiguous diagnostic results. To these stakeholders, accurate PCD diagnosis is of the utmost importance and is the first step towards successfully managing their PCD in the long-term. Research has demonstrated that other PCD patients feel the same as our patient representatives, with many</td>
<td>The panel which included patients' representatives made the following assumptions about the patient-important outcomes:

<table><tr><th>Outcomes</th><th>Relative importance</th></tr><tr><td>Premature death</td><td>CRITICAL</td></tr><tr><td>Need for lung transplant</td><td>CRITICAL</td></tr></table></td>
</tr>
</table>

	variability ○ Probably no important uncertainty or variability **○ No important uncertainty or variability**	harboring distrust of the medical system over the uncertainty surrounding their PCD diagnosis. Patients also report feeling stigmatized and embarrassed due to long-term uncertainty over their PCD diagnosis (37).	Lobectomy CRITICAL Rapid deterioration of pulmonary function CRITICAL Restriction in physical functioning or activity CRITICAL Development of bronchiectasies CRITICAL Deterioration of quality of life CRITICAL Recurrent sinopulmonary exacerbations CRITICAL Recurrent hospitalisations CRITICAL Hearing loss or speech delay CRITICAL Recurrent antibiotics use IMPORTANT Need for ear tube placement IMPORTANT Need for sinus surgery IMPORTANT Infertility IMPORTANT Anxiety related to delayed diagnosis IMPORTANT Side effects of repeat testing IMPORTANT Absenteeism IMPORTANT Poor social functioning IMPORTANT Resources use IMPORTANT
BALANCE OF EFFECTS	**Does the balance between desirable and undesirable effects favor the intervention or the comparison?** ○ Favors the comparison	The balance of direct desirable/undesirable effects favors the index test over the reference standard. False negative results, which are of critical importance in this analysis, are similar in frequency with nasal NO and the reference standard. Thus, the balance of downstream consequences does not favor either the index test or reference standard.	

	○ Probably favors the comparison ○ Does not favor either the intervention or the comparison **○ Probably favors the intervention** ○ Favors the intervention ○ Varies ○ Don't know		
RESOURCES REQUIRED	**How large are the resource requirements (costs)?** ○ Large costs ○ Moderate costs ○ Negligible costs and savings ○ Moderate savings ○ Large savings ○ Varies **○ Don't know**		While the per-test cost of nNO is relatively low, the cost of the chemiluminescent device is considerable, and would typically only be purchased for nasal NO measurements. In comparison, most academic sites already own the necessary laboratory equipment for ciliary TEM and many sites send their ciliary biopsies to third party sites for TEM processing and analysis. Genetic testing does not require institutions to purchase any start-up materials. In addition, for nasal NO, there are costs associated with training lab personnel, and the device is not FDA approved for clinical use in the USA. Thus, currently hospitals are not able to bill for the nNO measurement procedure.

	nNO (cost of device)	**nNO (cost per test for consumables and labor)**	**TEM***	**Genetics ***
St Louis, Missouri, USA	$40,000	$85.00	$1,520	$950 (38)
Israel	$40,000	$30.00	$1,000	not provided
Southampton, UK	$40,000	not provided	$730	not provided
Montreal, Canada	$40,000	$25.00	$550	$950
Denver, Colorado, USA	$40,000	not provided	$715	$950
Meunster, Germany	$40,000	$38.00	$750	$2,900

All prices are presented in US dollars.

		*Assuming that the baseline equipment/device is already available within the hospitals offering the tests.	
CERTAINTY OF EVIDENCE OF REQUIRED RESOURCES	**What is the certainty of the evidence of resource requirements (costs)?** ○ Very low ○ Low ○ Moderate ○ High **○ No included studies**	All cost information was obtained from international expert PCD centers, through personal communications with center directors.	
COST EFFECTIVENESS	**Does the cost-effectiveness of the intervention favor the intervention or the comparison?** ○ Favors the comparison ○ Probably favors the comparison ○ Does not favor either the intervention or the comparison	No research evidence was identified.	While the per-test cost of nNO is far less than that of either TEM or genetics, the cost of the initial device purchase must be factored in. However, this purchase cost is borne by hospital institutions and not by the patients being tested, whereas the costs for clinical TEM and genetic testing are paid by patients and are considerably higher than nNO testing costs. Thus, the cost-effectiveness is likely variable, but may favor less expense for patients undergoing nNO testing.

	○ Probably favors the intervention ○ Favors the intervention ○ Varies ○ **No included studies**		
EQUITY	**What would be the impact on health equity?** ○ Reduced ○ Probably reduced ○ **Probably no impact** ○ Probably increased ○ Increased ○ Varies ○ Don't know	No research evidence was identified.	Both nNO and TEM require travel to a specialized center, whereas genetic testing does not. The financial implications are unclear due to variability in charges and reimbursements for different procedures.
ACCEPTABILITY	**Is the intervention acceptable to key stakeholders?** ○ No ○ Probably no ○ Probably yes ○ **Yes** ○ Varies ○ Don't know	No research evidence was identified.	Nasal NO is accurate, painless, produces immediate results and is relatively inexpensive to the consumer. Thus, PCD patients and families of PCD patients on this committee strongly approved of this intervention.
FEASIBILITY	**Is the intervention feasible to implement?**	No research evidence was identified.	Nasal NO testing requires purchase of a rather expensive device that is generally used solely for this PCD detection as well as specialized training

	○ No ○ Probably no ○ **Probably yes** ○ Yes ○ Varies ○ Don't know		of lab personnel. Thus, centers must have available resources and dedicated personnel to perform the testing. In addition, there is likely a minimum number of tests that should be performed annually in order to ensure competency, though that number is not known.

Summary of judgments – Question 2

	JUDGMENT							IMPLICATIONS
PROBLEM	No	Probably no	Probably yes	**Yes**		Varies	Don't know	
TEST ACCURACY	Very inaccurate	Inaccurate	Accurate	**Very accurate**		Varies	Don't know	
DESIRABLE EFFECTS	Trivial	Small	**Moderate**	Large		Varies	Don't know	
UNDESIRABLE EFFECTS	Large	Moderate	**Small**	Trivial		Varies	Don't know	
CERTAINTY OF THE EVIDENCE OF TEST ACCURACY	Very low	Low	**Moderate**	High			No included studies	
CERTAINTY OF THE EVIDENCE OF TEST'S EFFECTS	Very low	Low	Moderate	High			**No included studies**	
CERTAINTY OF THE EVIDENCE OF MANAGEMENT'S EFFECTS	Very low	**Low**	Moderate	High			No included studies	
CERTAINTY OF THE EVIDENCE OF TEST RESULT/MANAGEMENT	Very low	**Low**	Moderate	High			No included studies	

	JUDGMENT							IMPLICATIONS
CERTAINTY OF EFFECTS	**Very low**	Low	Moderate	High			No included studies	
VALUES	Important uncertainty or variability	Possibly important uncertainty or variability	Probably no important uncertainty or variability	**No important uncertainty or variability**				
BALANCE OF EFFECTS	Favors the comparison	Probably favors the comparison	Does not favor either the intervention or the comparison	**Probably favors the intervention**	Favors the intervention	Varies	Don't know	
RESOURCES REQUIRED	Large costs	Moderate costs	Negligible costs and savings	Moderate savings	Large savings	Varies	**Don't know**	
CERTAINTY OF EVIDENCE OF REQUIRED RESOURCES	Very low	Low	Moderate	High			**No included studies**	
COST EFFECTIVENESS	Favors the comparison	Probably favors the comparison	Does not favor either the intervention or the comparison	Probably favors the intervention	Favors the intervention	Varies	**No included studies**	
EQUITY	Reduced	Probably reduced	**Probably no impact**	Probably increased	Increased	Varies	Don't know	
ACCEPTABILITY	No	Probably no	Probably yes	**Yes**		Varies	Don't know	
FEASIBILITY	No	Probably no	**Probably yes**	Yes		Varies	Don't know	

Conclusions – Question 2

TYPE OF RECOMMENDATION	Strong recommendation against the intervention ○	Conditional recommendation against the intervention ○	Conditional recommendation for either the intervention or the comparison ○	Conditional recommendation for the intervention ○	**Strong recommendation for the intervention** ○	
RECOMMENDATION	In cooperative patients >5 years old, with a clinical phenotype consistent with PCD and with cystic fibrosis excluded, we recommend using nasal nitric oxide as a diagnostic test for PCD in conjunction with TEM or genetic testing.					
JUSTIFICATION	The direct desirable consequences of using nNO instead of the reference standards outweighed the undesirable consequences. The overall impact of avoiding direct costs, complications and burden of repeat testing justified using nNO testing as a replacement to reference standards. The overall rate of false negatives (which was considered critical) and false positives were small and thus the downstream consequences were considered similar between the two test strategies. Nevertheless, despite the reported high accuracy of nNO in comparison to the reference standards, the panel estimates that the former might be more sensitive than the latter, thus potentially reducing the false positive results and their downstream consequences. Furthermore, nNO testing was considered acceptable to key stakeholders and possibly feasible to implement.					
SUBGROUP CONSIDERATIONS	This recommendation specifically applies to: 1) cooperative patients (generally over 5 years old) since nNO measurements can only be performed with the active participation of the individual being tested 2) Patients with a high probability of having PCD based on a compatible clinical phenotype (after ruling out cystic fibrosis) since a low pre-test probability would significantly increase the likelihood of false positive results, then making this test inappropriate as a replacement for the reference standards.					
IMPLEMENTATION CONSIDERATIONS	Chemiluminescent nitric oxide analyzers are expensive to initially purchase ($40,000 USD), yet the cost in consumable equipment and labor per test is quite reasonable (<$85 USD). While clinical centers have to absorb the purchase cost of nitric oxide devices, patients and the medical system should see a cost reduction for overall PCD diagnostic testing as more TEM and genetic tests are avoided in patients with normal nNO levels. Centers must also routinely train laboratory personnel in standard operating procedures for nNO measurement, which may add additional costs to implementing nNO testing.					
MONITORING AND EVALUATION	Centers performing nNO analysis for PCD diagnosis must follow strict standard operating procedures for nNO measurement and ensure technicians performing nNO testing are adequately trained in this technique. The PCD Foundation has nNO testing protocols, and centers performing nNO measurement may contact the PCD Foundation for site accreditation.					

RESEARCH PRIORITIES	Randomized trials evaluating all possible strategies and patient-important outcomes should be performed. The value of nNO testing in the face of the new extended PCD genetic panels will have to be confirmed through ongoing studies.

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Figure E3.1: PRISMA Flow diagram for Question 3

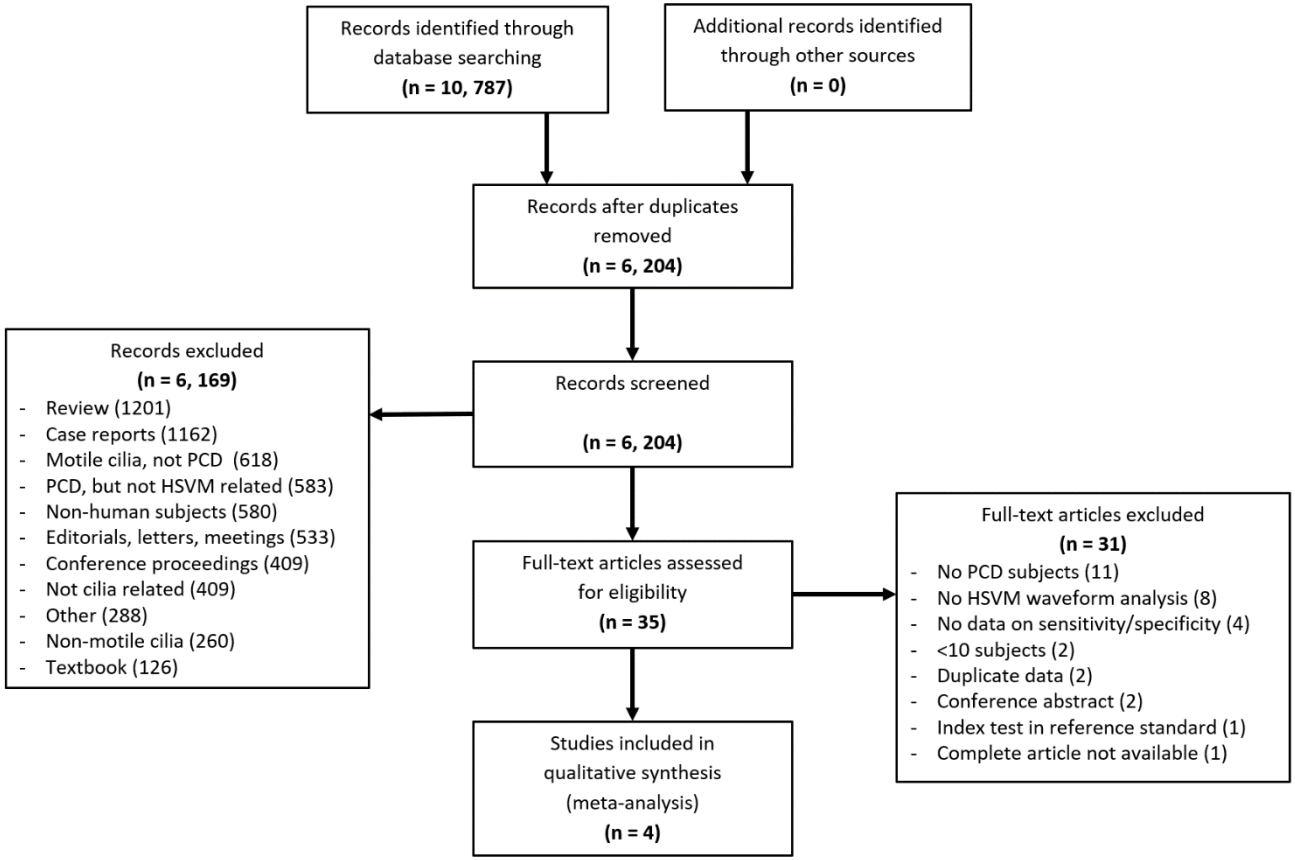
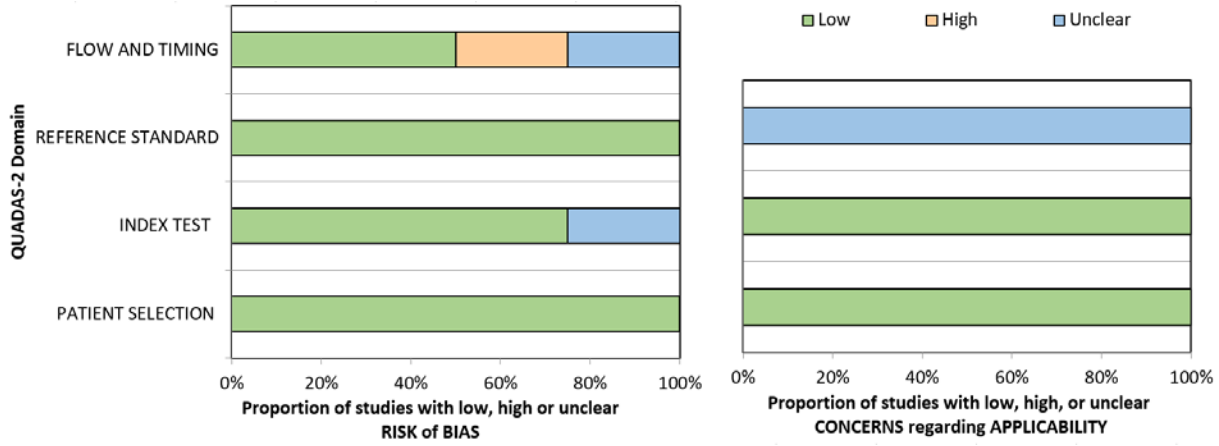


Figure E3.2: Quality assessment of individual studies with QUADAS-2 for Question 3



	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Hirst 2010	Low	Unclear	Low	High
Hirst 2014	Low	Low	Low	Unclear
Papon 2012	Low	Low	Low	Low
Stannard 2010	Low	Low	Low	Low

	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Hirst 2010	Low	Low	Unclear
Hirst 2014	Low	Low	Unclear
Papon 2012	Low	Low	Unclear
Stannard 2010	Low	Low	Unclear

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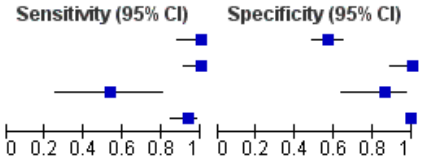
4. Stannard WA, Chilvers MA, Rutman AR, Williams CD, O'Callaghan C. Diagnostic testing of patients suspected of primary ciliary dyskinesia. *Am J Respir Crit Care Med* 2010; 181: 307-314.

Figure E3.3: Forest plot of included articles for Question 3

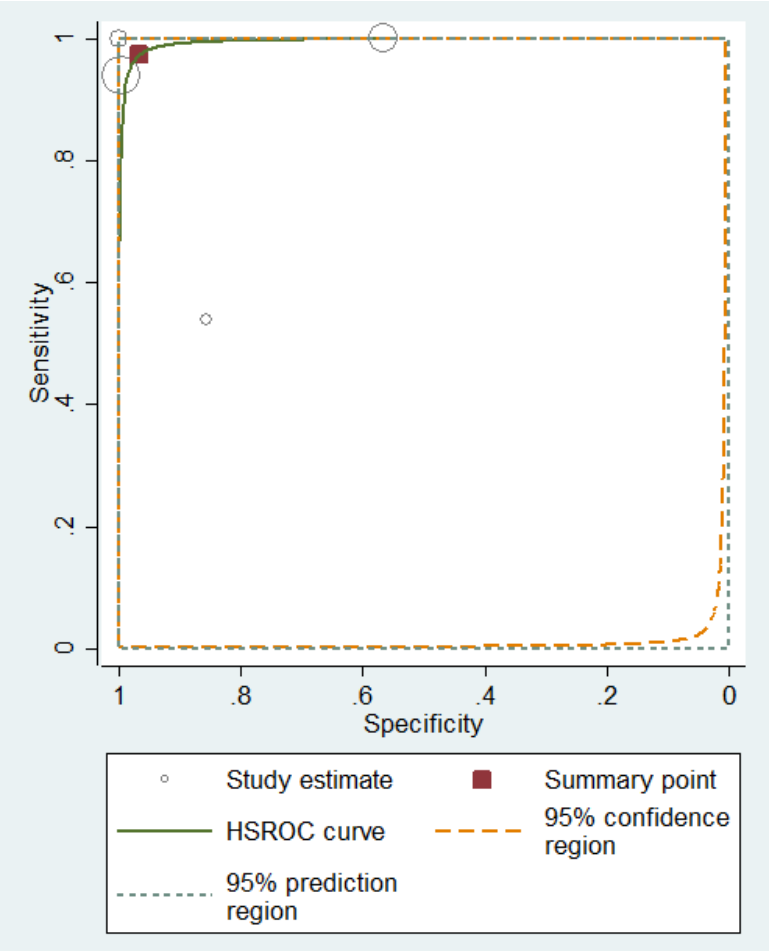
Study	TP	FP	FN	TN	Technique	Sensitivity (95% CI)	Specificity (95% CI)
Hirst 2010	28	69	0	90	CBP	1.00 [0.88, 1.00]	0.57 [0.49, 0.64]
Hirst 2014	41	0	0	32	CBP	1.00 [0.91, 1.00]	1.00 [0.89, 1.00]
Papon 2012	7	3	6	18	Dyskinesia Score	0.54 [0.25, 0.81]	0.86 [0.64, 0.97]
Stannard 2010	61	1	4	244	Dyskinesia Score	0.94 [0.85, 0.98]	1.00 [0.98, 1.00]



Abbreviations: CI – confidence interval, CBP – ciliary beat pattern, TP – true positive, FP – false positive, FN – false negative, TN – true negative

1. Hirst RA, Rutman A, Williams G, O'Callaghan C. Ciliated air-liquid cultures as an aid to diagnostic testing of primary ciliary dyskinesia. *Chest* 2010; 138: 1441-1447.
2. Hirst RA, Jackson CL, Coles JL, Williams G, Rutman A, Goggin PM, Adam EC, Page A, Evans HJ, Lackie PM, O'Callaghan C, Lucas JS. Culture of primary ciliary dyskinesia epithelial cells at air-liquid interface can alter ciliary phenotype but remains a robust and informative diagnostic aid. *PLoS ONE* 2014; 9: e89675.
3. Papon JF, Bassinet L, Cariou-Patron G, Zerah-Lancner F, Vojtek AM, Blanchon S, Crestani B, Amselem S, Coste A, Housset B, Escudier E, Louis B. Quantitative analysis of ciliary beating in primary ciliary dyskinesia: a pilot study. *Orphanet J Rare Dis* 2012; 7: 78.
4. Stannard WA, Chilvers MA, Rutman AR, Williams CD, O'Callaghan C. Diagnostic testing of patients suspected of primary ciliary dyskinesia. *Am J Respir Crit Care Med* 2010; 181: 307-314.

Figure E3.4: Summary ROC for Question 3



Summary receiver operating characteristic curve (ROC) for the 4 included studies.

1. Hirst RA, Rutman A, Williams G, O'Callaghan C. Ciliated air-liquid cultures as an aid to diagnostic testing of primary ciliary dyskinesia. *Chest* 2010; 138: 1441-1447.
2. Hirst RA, Jackson CL, Coles JL, Williams G, Rutman A, Goggin PM, Adam EC, Page A, Evans HJ, Lackie PM, O'Callaghan C, Lucas JS. Culture of primary ciliary dyskinesia epithelial cells at air-liquid interface can alter ciliary phenotype but remains a robust and informative diagnostic aid. *PLoS ONE* 2014; 9: e89675.
3. Papon JF, Bassinet L, Cariou-Patron G, Zerah-Lancner F, Vojtek AM, Blanchon S, Crestani B, Amselem S, Coste A, Housset B, Escudier E, Louis B. Quantitative analysis of ciliary beating in primary ciliary dyskinesia: a pilot study. *Orphanet J Rare Dis* 2012; 7: 78.
4. Stannard WA, Chilvers MA, Rutman AR, Williams CD, O'Callaghan C. Diagnostic testing of patients suspected of primary ciliary dyskinesia. *Am J Respir Crit Care Med* 2010; 181: 307-314.

Table E3.1: Summary of findings table for Question 3

Sensitivity	0.97 (95% CI: 0.60 to 1.00)
Specificity	0.96 (95% CI: 0.64 to 1.00)

Prevalence	35% (1)

Outcome	№ of studies (№ of patients)	Study design	Factors that may decrease quality of evidence					Effect per 100 patients tested	Test accuracy QoE	Importance
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 35%		
True positives (patients with PCD)	4 studies, 147 patients	cross-sectional (cohort type accuracy study)	not serious	not serious	serious [a]	serious [b]	none	34 (21 to 35)	⊕⊕◯◯ LOW	Critical
False negatives (patients incorrectly classified as not having PCD)								1 (0 to 14)		Critical
True negatives (patients without PCD)	4 studies, 457 patients	cross-sectional (cohort type accuracy study)	not serious	not serious	serious [a]	serious [b]	none	63 (41 to 65)	⊕⊕◯◯ LOW	Critical
False positives (patients incorrectly classified as having PCD)								2 (0 to 24)		Important

CI – confidence interval, QoE – quality of evidence

a. Accuracy estimates vary greatly across studies (a variation that would very likely lead to alternative diagnostic approaches) with confidence intervals frequently not overlapping.
b. One study included a very small number of patients and reported very wide confidence intervals (Papon 2012) in the context of an analysis including a small number of studies (extreme boundaries would very likely lead to alternative diagnostic approaches).

1. Leigh MW, Ferkol TW, Davis SD, Lee HS, Rosenfeld M, Dell SD, Sagel SD, Milla C, Olivier KN, Sullivan KM, Zariwala MA, Pittman JE, Shapiro AJ, Carson JL, Krischer J, Hazucha MJ, Knowles MR. Clinical Features and Associated Likelihood of Primary Ciliary Dyskinesia in Children and Adolescents. *Ann Am Thorac Soc* 2016; 13: 1305-1313.
2. Hirst RA, Rutman A, Williams G, O'Callaghan C. Ciliated air-liquid cultures as an aid to diagnostic testing of primary ciliary dyskinesia. *Chest* 2010; 138: 1441-1447.
3. Hirst RA, Jackson CL, Coles JL, Williams G, Rutman A, Goggin PM, Adam EC, Page A, Evans HJ, Lackie PM, O'Callaghan C, Lucas JS. Culture of primary ciliary dyskinesia epithelial cells at air-liquid interface can alter ciliary phenotype but remains a robust and informative diagnostic aid. *PLoS ONE* 2014; 9: e89675.
4. Papon JF, Bassinet L, Cariou-Patron G, Zerah-Lancner F, Vojtek AM, Blanchon S, Crestani B, Amselem S, Coste A, Housset B, Escudier E, Louis B. Quantitative analysis of ciliary beating in primary ciliary dyskinesia: a pilot study. *Orphanet J Rare Dis* 2012; 7: 78.
5. Stannard WA, Chilvers MA, Rutman AR, Williams CD, O'Callaghan C. Diagnostic testing of patients suspected of primary ciliary dyskinesia. *Am J Respir Crit Care Med* 2010; 181: 307-314.

Table E3.2 Evidence to Decision Table - Question 3

Should digital high speed videomicroscopy with ciliary beat pattern analysis alone be used as a PCD diagnostic test, in adult and pediatric patients, who are at high probability of having PCD (as a replacement of reference standards of classic TEM structural ciliary defect or biallelic causative mutations in PCD genes)?

POPULATION:	Patients with a high pre-test probability	**BACKGROUND:** Digital high speed videomicroscopy with ciliary beat pattern analysis (HSVM) has been used in a number of specialized laboratories to diagnose PCD (32, 69, 70, 76). Using a digital high speed video camera attached to a microscope, beating ciliated epithelial edges are recorded at frame rates of between 120–500 frames per second (fps) and are then replayed at slower rates to view ciliary motion. Samples can then be evaluated to assess ciliary function by measuring cilia beat frequency (CBF) and/or cilia beat pattern (CBP). Recent expert consensus recommended HVSM ciliary functional assessment of both CBF and CBP coupled with TEM as a means of diagnosing PCD (77). However, conducting HSVM proves challenging, requiring significant expertise and training. Furthermore, this expertise is limited to a few laboratories in Europe and Canada; therefore, restricting clinical applicability.
INTERVENTION:	Digital high speed videomicroscopy with ciliary beat pattern analysis	
PURPOSE OF THE TEST:	Diagnosis of PCD	
LINKED TREATMENTS:	Targeted pulmonary/ENT care in a PCD specialized center in patients with confirmed PCD or further investigations for other potentially treatable diseases in patients with negative testing for PCD	
ANTICIPATED OUTCOMES:	Premature death, need for lung transplant, rapid deterioration of pulmonary function, restriction in physical functioning/activity, development of bronchiectasis, deterioration of overall quality of life, recurrent sinopulmonary exacerbations, recurrent hospitalizations, hearing loss or speech delay, recurrent antibiotics use, need for ear tube placement, need for sinus surgery, infertility, depression/anxiety and side effects of repeat testing, absenteeism, poor social functioning, resources use	
SETTING:	Outpatient setting	
PERSPECTIVE:	Clinical recommendation from an individual perspective	

Assessment – Question 3

<table>
<tr><th></th><th>JUDGMENT</th><th>RESEARCH EVIDENCE</th><th>ADDITIONAL CONSIDERATIONS</th></tr>
<tr><td>PROBLEM</td><td>Is the problem a priority?
○ No
○ Probably no
○ Probably yes
○ Yes

○ Varies
○ Don't know</td><td>Many clinical centers across Europe rely upon digital HSVM as their primary diagnostic test for PCD (1), yet inter-rater agreement of HSVM analysis is quite poor, even in samples from healthy controls (2). Current recommendations call for repeat biopsies on multiple occasions or cellular regrowth of ciliated sample to insure permanence of diagnostic abnormalities (i.e. not due to secondary insults, such as viral infection or pollutant exposure) (3). The use of this test has the potential to result in false positive and false negative PCD diagnoses.</td><td></td></tr>
<tr><td>TEST ACCURACY</td><td>How accurate is the test?
○ Very inaccurate
○ Inaccurate
○ Accurate
○ Very accurate

○ Varies
○ Don't know</td><td>Sensitivity of HSVM was 97% (95% CI 60-100) and specificity was 96% (64-100) in a population with a pre-test probability of 35%.
<table><tr><th>Test results</th><th>Importance</th><th>Effects per 100 patients tested</th><th>Quality of evidence</th></tr><tr><td>TP</td><td>Critical</td><td>34 (21 to 35)</td><td rowspan="2">⊕⊕◯◯
LOW</td></tr><tr><td>FN</td><td>Critical</td><td>1 (0 to 14)</td></tr><tr><td>TN</td><td>Critical</td><td>63 (41 to 65)</td><td rowspan="2">⊕⊕◯◯
LOW</td></tr><tr><td>FP</td><td>Important</td><td>2 (0 to 24)</td></tr></table></td><td>Since genetic testing was not used in the reference standard of the analyzed studies, the sensitivity is possibly overestimated. Also, as HSVM is only performed in a single, highly specialized center in 3 out of 4 analyzed studies, it is possible that both sensitivity and accuracy is overestimated.</td></tr>
<tr><td>DESIRABLE EFFECTS</td><td>How substantial are the desirable anticipated effects?
○ Trivial
○ Small
○ Moderate
○ Large

○ Varies
○ Don't know</td><td><table><tr><th></th><th>Index test +
(Abnormal HSVM)</th><th>Index test –
(Normal HSVM)</th></tr></table></td><td>*The panel considered that the undesirable downstream consequences of false positive results are difficult to assess and thus uncertain for 2 main reasons: 1) false positive results could still be PCD since ongoing studies are showing that the references standards of TEM and genetic testing lack sensitivity to detect PCD (i.e. new genetic variants are discovered each year) 2) great heterogeneity in the non-PCD true</td></tr>
</table>

UNDESIRABLE EFFECTS	**How substantial are the undesirable anticipated effects?** ○ Large ○ **Moderate** ○ Small ○ Trivial ○ Varies ○ Don't know	**PCD +**	TRUE POSITIVES -Referral to a PCD specialized center -Rapid cessation of repeat testing, thus avoid unnecessary supplementary costs and anxiety over awaiting confirmation of PCD diagnosis -PCD targeted pulmonary and ENT therapies with probable clinical improvement	FALSE NEGATIVES ** -May still have PCD as not all forms of PCD result in abnormal HSVM. -Discharge from a PCD specialized center (*diagnosis of PCD will likely be missed*) -Unnecessary investigation for other diseases -Unnecessary supplementary costs and anxiety over awaiting diagnosis -No PCD targeted pulmonary and ENT care, and may receive other non-PCD cares with risks (e.g. IVIG with blood product exposures, lobectomy)	underlying disease thus the expected effects of the PCD targeted pulmonary and ENT therapies. **The panel considered that the undesirable downstream consequences of false negative results difficult to assess and thus uncertain for 2 main reasons: 1) the effect could be have been underestimated since the studies assessing the impact of delayed diagnosis were not recently performed, and the standard of care has greatly improved (as well as the patient outcomes), 2) the effect could have been overestimated since older age at PCD diagnosis (usually correlated with delayed diagnosis) is associated with distrust in medical community, with less improvement in the St George's Respiratory Questionnaire scores, worsened long-term compliance with PCD treatment regimens (4) and ultimately, with worse outcomes (increased rates of respiratory cultures positive for *Pseudomonas aeruginosa* infection (5), which causes worse outcomes in similar respiratory diseases (6), increased rates of medical and surgical complications, including nasal polyposis, hemoptysis, and lobectomy surgery, all of which can cause significant morbidity and even mortality (7)).
		PCD -	FALSE POSITIVES * -Referral to a PCD specialized center (*diagnosis of the true disease will likely be delayed*) -PCD targeted pulmonary and ENT care with possible clinical improvement regardless of the cause of chronic lung disease. -No specific therapy for the true underlying disease, if it exists (e.g.	TRUE NEGATIVES -Discharge from a PCD specialized center -Investigation for other potentially treatable diseases (such as immunodeficiency) -Rapid cessation of repeat testing, thus avoid unnecessary supplementary costs and anxiety over awaiting information of PCD diagnosis	

		IVIG for immunodeficiency)	
CERTAINTY OF THE EVIDENCE OF TEST ACCURACY	**What is the overall certainty of the evidence of test accuracy?** ○ Very low ○ **Low** ○ Moderate ○ High ○ No included studies	Imprecision and inconsistency across included studies led to decreased rating of the certainty in the evidence. Detailed judgment is provided in the evidence profiles tables.	
CERTAINTY OF THE EVIDENCE OF TEST'S EFFECTS	**What is the overall certainty of the evidence for any critical or important direct benefits, adverse effects or burden of the test?** ○ Very low ○ Low ○ Moderate ○ High ○ **No included studies**	No direct evidence for critical or important direct benefits, adverse effects or burden of the test (i.e. side effects of repeat testing and anxiety related to delayed diagnosis) was considered here.	The panel assumed that: 1) HSVM analysis requires patients to travel to experienced centers on at least three separate occasions, and results are delayed due to the lengthy interpretation times (3). If only one biopsy sample is obtained, and cells are regrown in culture, this will avoid repeat travel by the patient, but the time to result will be several months. Interpretation is labor intensive, as there is no specialized software for automated interpretation. Complications of biopsy are minimal (mild discomfort, possibly mild bleeding).

			2) TEM analysis requires patients to travel to experienced centers, can take weeks to produce results, can result in non-diagnostic results requiring repeat biopsy, and complications of biopsy are minimal (mild discomfort, possibly mild bleeding) (8, 9). 3) Genetic testing does not require patient travel, can take weeks to produce results, complications of venipuncture are minimal (mild discomfort), and can result in non-diagnostic results with variants of unknown significance (10), requiring other PCD diagnostic tests. If TEM and/or genetics are replaced by HSVM analysis, patients will need to travel to specialized centers for biopsy and HSVM analysis. Often, travel will be required on 3 separate occasions, as is recommended with functional ciliary analysis, versus weeks of ciliated cell regrowth in culture with HSVM analysis afterwards (3). There are only minimal direct differences between the direct desirable and undesirable effects of the index test of HSVM analysis and the reference standards of TEM and/or genetic testing. Thus, neither the index test or reference standards are favored over one another.
CERTAINTY OF THE EVIDENCE OF	**What is the overall certainty of the evidence of effects of the management that is guided by the test results?** ○ Very low ○ **Low**	No direct evidence comparing PCD targeted pulmonary and ENT care versus no treatment was considered since these treatments consist of a bundle of different supportive therapies which are usually at least partially started for symptom relief. Nevertheless, longitudinal PCD studies show that patient using long term standard PCD regimens experienced less decline in lung function than patients left undiagnosed and thus untreated	The panel considered that standard PCD therapies are likely more efficient than what is currently reported, but equipoise would preclude studying the natural evolution of the disease without minimal intervention.

	○ Moderate ○ High ○ No included studies	(11-13). Referral of pediatric patients to a PCD center of excellence for long-term therapies may also improve lung function and nutrition (14). Furthermore, later diagnosis (in adulthood) of PCD might be linked to worsened long-term pulmonary outcomes (11). Other individual interventions were occasionally studied but could not be pooled due to the heterogeneity of interventions and/or comparators for each critical outcome. For instance, children with PCD and chronic otitis media with effusion show marked improvements in hearing after surgical placement of ventilation tubes versus medical therapy alone (15, 16). Aggressive surgical management of chronic rhinosinusitis in PCD patients also provides significant symptom relief (17). Regular airway clearance also shows improvements in lung function in one small cross-over RCT (18).	
CERTAINTY OF THE EVIDENCE OF TEST RESULT/MANAGEMENT	**How certain is the link between test results and management decisions?** ○ **Very low** ○ Low ○ Moderate ○ High ○ No included studies	Observational studies showed that PCD patients will promptly begin standard therapies for PCD, including daily airway clearance, sputum culture surveillance, otolaryngology care, and aggressive use of antibiotics for respiratory infections (1, 19). Nevertheless, these therapies may be suboptimal outside of PCD specialized centers. Furthermore, erratic long-term compliance with PCD treatment regimens, especially in older patients at diagnosis (4), increases uncertainty regarding the link between testing and treatment.	The panel confirms that in clinical practice a positive diagnostic for PCD will almost certainly lead to chronic therapies if patient is referred to a PCD specialized center.
CERTAINTY OF EFFECTS	**What is the overall certainty of the evidence of effects of the test?** ○ **Very low** ○ Low ○ Moderate ○ High	The overall certainty of the evidence of the effects of testing and subsequent management decisions on patient-important outcomes is limited by the very low certainty regarding the link between results and management decisions and the low certainty of the effects of the management guided by the test results.	

	○ No included studies		
VALUES	**Is there important uncertainty about or variability in how much people value the main outcomes?** ○ Important uncertainty or variability ○ Possibly important uncertainty or variability ○ Probably no important uncertainty or variability **○ No important uncertainty or variability**	There are also numerous publications addressing the stress created in patients surrounding their difficulty obtaining a proper PCD diagnosis. Indeed, uncertainty surrounding PCD diagnosis has been linked to poor psychosocial outcomes (20, 21). Several PCD patients and family representatives of PCD patients sat on this committee, and they repeatedly voiced their frustration with poor quality diagnostic testing and ambiguous diagnostic results. To these stakeholders, accurate PCD diagnosis is of the utmost importance and is the first step towards successfully managing their PCD in the long-term. Research has demonstrated that other PCD patients feel the same as our patient representatives, with many harboring distrust of the medical system over the uncertainty surrounding their PCD diagnosis. Patients also report feeling stigmatized and embarrassed due to long-term uncertainty over their PCD diagnosis (22).	The panel which included patients' representatives made the following assumptions about the patient-important outcomes: **Outcomes** / **Relative importance** Premature death — CRITICAL Need for lung transplant — CRITICAL Lobectomy — CRITICAL Rapid deterioration of pulmonary function — CRITICAL Restriction in physical functioning/activity — CRITICAL Development of bronchiectasies — CRITICAL Deterioration of quality of life — CRITICAL Recurrent sinopulmonary exacerbations — CRITICAL Recurrent hospitalisations — CRITICAL Hearing loss or speech delay — CRITICAL Recurrent antibiotics use — IMPORTANT Need for ear tube placement — IMPORTANT Need for sinus surgery — IMPORTANT Infertility — IMPORTANT

			Anxiety related to delayed diagnosis — IMPORTANT Side effects of repeat testing — IMPORTANT Absenteeism — IMPORTANT Poor social functioning — IMPORTANT Resources use — IMPORTANT
BALANCE OF EFFECTS	**Does the balance between desirable and undesirable effects favor the intervention or the comparison?** ○ Favors the comparison ○ **Probably favors the comparison** ○ Does not favor either the intervention or the comparison ○ Probably favors the intervention ○ Favors the intervention ○ Varies ○ Don't know	False negative results, which are of critical importance in this analysis, are relatively more frequent with HSVM testing. However, false positive results, which are important but not critical, may be increased if patients only undergo HSVM testing on one occasion. Repeat HSVM testing or cellular regrowth should greatly decrease false positive results. Thus, the balance of indirect benefits/harms probably favors the reference standard.	

<table>
<tr>
<td>RESOURCES REQUIRED</td>
<td>How large are the resource requirements (costs)?
○ Large costs
○ Moderate costs
○ Negligible costs and savings
○ Moderate savings
○ Large savings

○ Varies
○ Don't know</td>
<td>
<table>
<tr><th></th><th>HSVM testing** X3</th><th>HSVM testing** with cell culture</th><th>TEM*</th><th>Genetics*</th></tr>
<tr><td>St Louis, Missouri, USA</td><td>Not performed</td><td>N/A</td><td>$1,520</td><td>$950 (23)</td></tr>
<tr><td>Israel</td><td>Not provided</td><td>N/A</td><td>$1,000</td><td>not provided</td></tr>
<tr><td>Southampton, UK</td><td>$4,410</td><td>$2,200</td><td>$730</td><td>not provided</td></tr>
<tr><td>Montreal, Canada</td><td>Not performed</td><td>N/A</td><td>$550</td><td>$950</td></tr>
<tr><td>Denver, Colorado, USA</td><td>Not performed</td><td>N/A</td><td>$715</td><td>$950</td></tr>
<tr><td>Meunster, Germany</td><td>$360</td><td>$495</td><td>$750</td><td>$2,900</td></tr>
</table>
All prices are presented in US dollars.
*Assuming that the baseline equipment/device is already available within the hospitals offering the tests.
**The purchase price of high speed digital recording equipment is approximately $50,000 US dollars.
</td>
<td>HSVM measurement requires sites to purchase expensive recording devices for analysis, which increases costs considerably. In comparison, most academic sites already own the necessary laboratory equipment for ciliary TEM, and many sites send their ciliary biopsies to third party sites for TEM processing and analysis. Genetic testing does not require institutions to purchase any start-up materials.</td>
</tr>
<tr>
<td>CERTAINTY OF EVIDENCE OF REQUIRED RESOURCES</td>
<td>What is the certainty of the evidence of resource requirements (costs)?
○ Very low
○ Low</td>
<td>All cost information was obtained from international expert PCD centers, through personal communications with center directors.</td>
<td></td>
</tr>
</table>

	○ Moderate ○ High **○ No included studies**		
COST EFFECTIVENESS	**Does the cost-effectiveness of the intervention favor the intervention or the comparison?** ○ Favors the comparison ○ Probably favors the comparison ○ Does not favor either the intervention or the comparison ○ Probably favors the intervention ○ Favors the intervention ○ Varies **○ No included studies**	No research evidence was identified.	Including the indirect costs of each PCD test above, there is not a large difference in prices for TEM, genetic, or HSVM testing to diagnose PCD. Out of pocket expenses for patients are likely higher for travel with repeat HSVM analysis, but this can be decreased by one biopsy with cellular culture. Patients do not have to pay for any travel expenses with genetic testing.
EQUITY	**What would be the impact on health equity?** ○ Reduced ○ Probably reduced **○ Probably no**	No research evidence was identified.	For patients living in remote areas, without easy access to specialized PCD centers, HSVM testing would not add any convenience and would likely be infeasible. For patients without medical insurance, the cost of repeat clinical visits with HSVM testing, or HSVM analysis after

	impact ○ Probably increased ○ Increased ○ Varies ○ Don't know		cellular re-growth, is greater than TEM or genetic testing. However, in some locations, this cost difference is quite small.
QU	**Is the intervention acceptable to key stakeholders?** ○ **No** ○ Probably no ○ Probably yes ○ Yes ○ Varies ○ Don't know	The intervention of HSVM analysis as a diagnostic test for PCD lacks accuracy outside of only a few highly specialized centers. Only two labs in the United Kingdom have published data illustrating successful implementation of HSVM in clinical practice (24-26). When expanded to other labs, the diagnostic accuracy drops significantly (27), and inter-rater agreement is poor, even in samples from healthy controls (2).	This decreased accuracy, outside of only a few labs worldwide, greatly concerns stakeholders, and for this reason, they do not accept HSVM testing as a reliable PCD diagnostic test.
FEASIBILITY	**Is the intervention feasible to implement?** ○ No ○ **Probably no** ○ Probably yes ○ Yes ○ Varies ○ Don't know	The intervention of HSVM analysis as a diagnostic test for PCD lacks accuracy outside of only a few highly specialized centers. Only two labs in the United Kingdom have published data illustrating successful implementation of HSVM in clinical practice (24, 25). When expanded to other labs, the diagnostic accuracy of HSVM analysis drops significantly (27). One highly experienced HSVM lab in Canada (including members formerly from UK-based labs) has shown poor inter-rater agreement of HSVM interpretation in samples from healthy controls (2).	For these reasons, HSVM testing is not a feasible intervention to employ across various clinical centers.

Summary of judgments – Question 3

	JUDGMENT							IMPLICATIONS
PROBLEM	No	Probably no	Probably yes	**Yes**		Varies	Don't know	

	JUDGMENT							IMPLICATIONS
TEST ACCURACY	Very inaccurate	**Inaccurate**	Accurate	Very accurate		Varies	Don't know	
DESIRABLE EFFECTS	Trivial	**Small**	Moderate	Large		Varies	Don't know	
UNDESIRABLE EFFECTS	Large	**Moderate**	Small	Trivial		Varies	Don't know	
CERTAINTY OF THE EVIDENCE OF TEST ACCURACY	Very low	**Low**	Moderate	High			No included studies	
CERTAINTY OF THE EVIDENCE OF TEST'S EFFECTS	Very low	Low	Moderate	High			**No included studies**	
CERTAINTY OF THE EVIDENCE OF MANAGEMENT'S EFFECTS	Very low	**Low**	Moderate	High			No included studies	
CERTAINTY OF THE EVIDENCE OF TEST RESULT/MANAGEMENT	**Very low**	Low	Moderate	High			No included studies	
CERTAINTY OF EFFECTS	**Very low**	Low	Moderate	High			No included studies	
VALUES	Important uncertainty or variability	Possibly important uncertainty or variability	Probably no important uncertainty or variability	**No important uncertainty or variability**				
BALANCE OF EFFECTS	Favors the comparison	**Probably favors the comparison**	Does not favor either the intervention or the comparison	Probably favors the intervention	Favors the interventio n	Varies	Don't know	

	JUDGMENT							IMPLICATIONS
RESOURCES REQUIRED	Large costs	Moderate costs	Negligible costs and savings	Moderate savings	Large savings	Varies	**Don't know**	
CERTAINTY OF EVIDENCE OF REQUIRED RESOURCES	Very low	Low	Moderate	High			**No included studies**	
COST EFFECTIVENESS	Favors the comparison	Probably favors the comparison	Does not favor either the intervention or the comparison	Probably favors the intervention	Favors the interventio n	Varies	**No included studies**	
EQUITY	Reduced	Probably reduced	**Probably no impact**	Probably increased	Increased	Varies	Don't know	
ACCEPTABILITY	**No**	Probably no	Probably yes	Yes		Varies	Don't know	
FEASIBILITY	No	**Probably no**	Probably yes	Yes		Varies	Don't know	

Conclusions – Question 3

Should digital high speed videomicroscopy with ciliary beat pattern analysis alone be used as a PCD diagnostic test, in adult and pediatric patients, who are at high probability of having PCD (as a replacement of reference standards of classic TEM structural ciliary defect or biallelic causative mutations in PCD genes)?

TYPE OF RECOMMENDATION	Strong recommendation against the intervention ○	**Conditional recommendation against the intervention** ○	Conditional recommendation for either the intervention or the comparison ○	Conditional recommendation for the intervention ○	Strong recommendation for the intervention ○
RECOMMENDATION	We suggest not using ciliary beat pattern analysis by HSVM as a replacement diagnostic test in patients who are at high probability of having PCD.				
JUSTIFICATION	Significant technical expertise and equipment is required to successfully conduct HSVM analysis. There is also a lack of standardization in HSVM interpretation techniques, with some centers using various quantitative functional measures based on qualitative assessments, while other centers use qualitative descriptions of beat pattern. With this lack of standardization in both sample preparation and interpretation, the HSVM technique itself is not easily transferred to other centers, and the applicability of the technique across centers currently remains poor. Only a few international centers have the expertise to conduct ciliary functional analysis with HSVM.				
SUBGROUP CONSIDERATIONS	NA				
IMPLEMENTATION CONSIDERATIONS	We do not recommend implementation of HSVM testing at this time.				
MONITORING AND EVALUATION	NA				
RESEARCH PRIORITIES	Standardization of HSVM protocols (including tissue culture conditions) and development of robust validated beat pattern measurements are required to pursue HSVM as a stand-alone PCD diagnostic test. To improve general applicability of HSVM, further research is indicated demonstrating that multiple centers can successfully use this tool when following validated standard operating protocols.				

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Figure E4.1: PRISMA Flow diagram for question 4

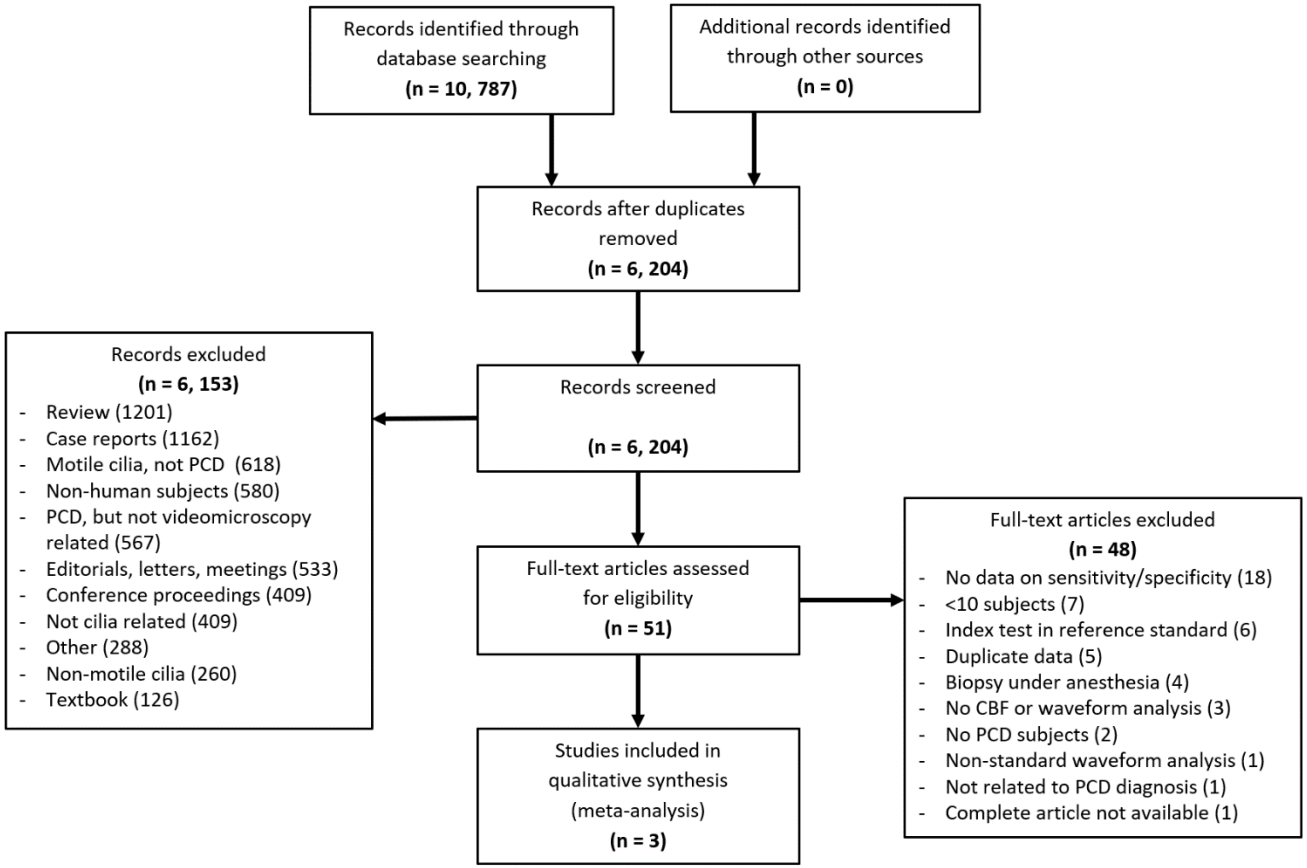
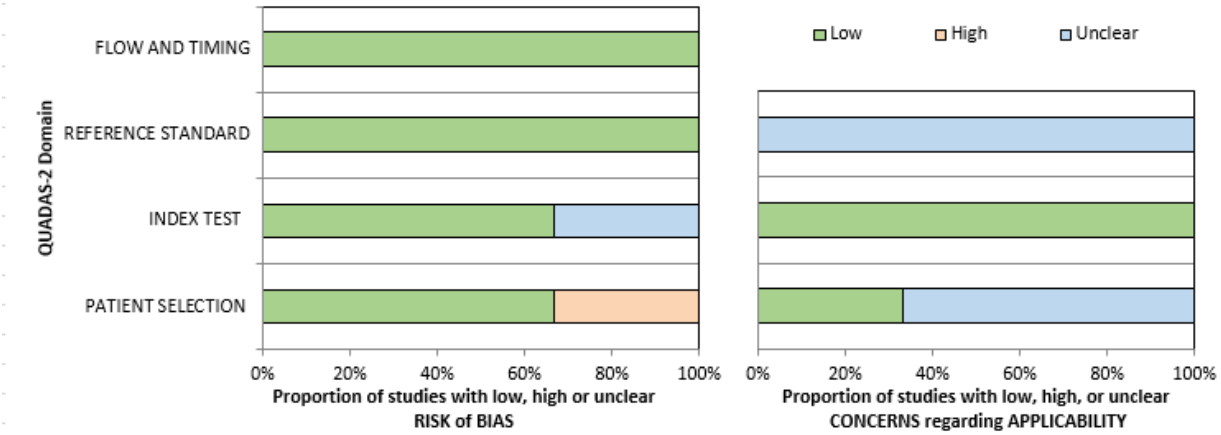


Figure E4.2: Quality assessment of individual studies with QUADAS-2 for Question 4



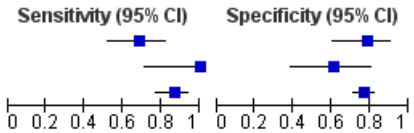
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING
Hirst 2014	Low	Unclear	Low	Low
Olm 2011	High	Low	Low	Low
Stannard 2010	Low	Low	Low	Low

	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Hirst 2014	Unclear	Low	Unclear
Olm 2011	Unclear	Low	Unclear
Stannard 2010	Low	Low	Unclear

1. Hirst RA, Jackson CL, Coles JL, Williams G, Rutman A, Goggin PM, Adam EC, Page A, Evans HJ, Lackie PM, O'Callaghan C, Lucas JS. Culture of primary ciliary dyskinesia epithelial cells at air-liquid interface can alter ciliary phenotype but remains a robust and informative diagnostic aid. *PLoS ONE* 2014; 9: e89675.
2. Olm MA, Kogler JE, Jr., Macchione M, Shoemark A, Saldiva PH, Rodrigues JC. Primary ciliary dyskinesia: evaluation using cilia beat frequency assessment via spectral analysis of digital microscopy images. *J Appl Physiol* 2011; 111: 295-302.
3. Stannard WA, Chilvers MA, Rutman AR, Williams CD, O'Callaghan C. Diagnostic testing of patients suspected of primary ciliary dyskinesia. *Am J Respir Crit Care Med* 2010; 181: 307-314.

Figure E4.3: Forest plot of included articles for Question 4

Study	TP	FP	FN	TN	Cutoff (Hz)	Sensitivity (95% CI)	Specificity (95% CI)
Hirst 2014	28	7	13	25	10.0	0.68 [0.52, 0.82]	0.78 [0.60, 0.91]
Olm 2011	11	9	0	14	10.0	1.00 [0.72, 1.00]	0.61 [0.39, 0.80]
Stannard 2010	61	62	9	208	11.0	0.87 [0.77, 0.94]	0.77 [0.72, 0.82]



Abbreviations: CI – confidence interval, TP – true positive, FP – false positive, FN – false negative, TN – true negative

1. Hirst RA, Jackson CL, Coles JL, Williams G, Rutman A, Goggin PM, Adam EC, Page A, Evans HJ, Lackie PM, O'Callaghan C, Lucas JS. Culture of primary ciliary dyskinesia epithelial cells at air-liquid interface can alter ciliary phenotype but remains a robust and informative diagnostic aid. *PLoS ONE* 2014; 9: e89675.
2. Olm MA, Kogler JE, Jr., Macchione M, Shoemark A, Saldiva PH, Rodrigues JC. Primary ciliary dyskinesia: evaluation using cilia beat frequency assessment via spectral analysis of digital microscopy images. *J Appl Physiol* 2011; 111: 295-302.
3. Stannard WA, Chilvers MA, Rutman AR, Williams CD, O'Callaghan C. Diagnostic testing of patients suspected of primary ciliary dyskinesia. *Am J Respir Crit Care Med* 2010; 181: 307-314.

Table E4.1: Summary of findings table for Question 4

Sensitivity	0.68 to 1.00
Specificity	0.61 to 0.78
Prevalence	35% (1)

Outcome	№ of studies (№ of patients)	Study design	Factors that may decrease quality of evidence					Effect per 100 patients tested	Test accuracy QoE	Importance
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	pre-test probability of 35%		
True positives (patients with PCD)	3 studies 122 patients	cohort & case-control type studies	not serious	not serious	serious [a]	serious [b]	none	24 to 35	⊕⊕◯◯ LOW	Important
False negatives (patients incorrectly classified as not having PCD)								0 to 11		Critical
True negatives (patients without PCD)	3 studies 325 patients	cohort & case-control type studies	not serious	not serious	serious [a]	serious [b]	none	40 to 51	⊕⊕◯◯ LOW	Important
False positives (patients incorrectly classified as having PCD)								14 to 25		Important

QoE – quality of evidence

a. Despite confidence intervals overlapping, accuracy estimates vary greatly between studies (a variation that would very likely lead to alternative diagnostic approaches).
b. One studies included a very small number of patients and reported wide confidence intervals (Olm 2011) in the context of an analysis including a very small number of studies (extreme boundaries would very likely lead to alternative diagnostic approaches).

1. Leigh MW, Ferkol TW, Davis SD, Lee HS, Rosenfeld M, Dell SD, Sagel SD, Milla C, Olivier KN, Sullivan KM, Zariwala MA, Pittman JE, Shapiro AJ, Carson JL, Krischer J, Hazucha MJ, Knowles MR. Clinical Features and Associated Likelihood of Primary Ciliary Dyskinesia in Children and Adolescents. *Ann Am Thorac Soc* 2016; 13: 1305-1313.
2. Hirst RA, Jackson CL, Coles JL, Williams G, Rutman A, Goggin PM, Adam EC, Page A, Evans HJ, Lackie PM, O'Callaghan C, Lucas JS. Culture of primary ciliary dyskinesia epithelial cells at air-liquid interface can alter ciliary phenotype but remains a robust and informative diagnostic aid. *PLoS ONE* 2014; 9: e89675.
3. Olm MA, Kogler JE, Jr., Macchione M, Shoemark A, Saldiva PH, Rodrigues JC. Primary ciliary dyskinesia: evaluation using cilia beat frequency assessment via spectral analysis of digital microscopy images. *J Appl Physiol* 2011; 111: 295-302.
4. Stannard WA, Chilvers MA, Rutman AR, Williams CD, O'Callaghan C. Diagnostic testing of patients suspected of primary ciliary dyskinesia. *Am J Respir Crit Care Med* 2010; 181: 307-314.

Table E4.2 Evidence to Decision Table - Question 4

Should ciliary beat frequency (CBF) or ciliary waveform analysis using light microscopy without high speed recording, be used as a PCD diagnostic test, in adult and pediatric patients, who are at high probability of having PCD (as replacement of reference standards of classic TEM structural ciliary defect and/or biallelic causative mutations in PCD genes)?		
POPULATION:	Patients with a high pre-test probability	**BACKGROUND:** Calculation of ciliary beat frequency (CBF) has been historically suggested as a PCD diagnostic method, which can be performed with inexpensive light microscopy and straightforward recording technology (1, 2). Additionally, some clinicians also employ ciliary waveform analysis without high-speed recording to diagnose PCD (3, 4). Some academic centers even suggest these tests as first line screening, and if results are normal, further PCD diagnostic testing (such as TEM or genetic testing) may not be necessary (5, 6). However, most expert North American PCD centers avoid CBF measurement or waveform analysis without high-speed recording in PCD, as several recently discovered genetic forms of PCD result in normal CBF with only subtle changes in ciliary waveform (7). In addition, most PCD researchers have migrated from standard speed video recording to high-speed videomicroscopy (HSVM), as this method provides more detailed ciliary waveform information for analysis.
INTERVENTION:	Ciliary Beat Frequency or ciliary waveform analysis using light microscopy without high speed recording	
PURPOSE OF THE TEST:	Diagnosis of PCD	
LINKED TREATMENTS:	Targeted pulmonary/ENT care in a PCD specialized center in patients with confirmed PCD or further investigations for other potentially treatable diseases in patients with negative testing for PCD	
ANTICIPATED OUTCOMES:	Premature death, need for lung transplant, rapid deterioration of pulmonary function, restriction in physical functioning/activity, development of bronchiectasis, deterioration of overall quality of life, recurrent sinopulmonary exacerbations, recurrent hospitalizations, hearing loss or speech delay, recurrent antibiotics use, need for ear tube placement, need for sinus surgery, infertility, depression/anxiety and side effects of repeat testing, absenteeism, poor social functioning, resources use	
SETTING:	Outpatient setting	
PERSPECTIVE:	Clinical recommendation from an individual perspective	

Assessment – Question 4

<table>
<tr><th></th><th>JUDGMENT</th><th>RESEARCH EVIDENCE</th><th>ADDITIONAL CONSIDERATIONS</th></tr>
<tr><td>PROBLEM</td><td>Is the problem a priority?
o No
o Probably no
o Probably yes
o Yes

o Varies
o Don't know</td><td>Some clinical centers across North America still employ inexpensive and rapid screening of ciliary motility by CBF on light microscopy before deciding to proceed to more labor and cost-intensive for definitive PCD diagnosis (TEM and/or genetics) (1, 2). The use of this test has the potential to result in false positive and false negative PCD diagnoses.</td><td></td></tr>
<tr><td>TEST ACCURACY</td><td>How accurate is the test?
o Very inaccurate
o Inaccurate
o Accurate
o Very accurate

o Varies
o Don't know</td><td>Sensitivity range varied from 68% to 100% and specificity range varied from 61% to 78% in a population with a pre-test probability of 35%.
<table>
<tr><th>Test results</th><th>Importance</th><th>Range of effects per 100 patients tested</th><th>Quality of evidence</th></tr>
<tr><td>TP</td><td>Important</td><td>24 to 35</td><td rowspan="2">⨁⨁◯◯
LOW</td></tr>
<tr><td>FN</td><td>Critical</td><td>0 to 11</td></tr>
<tr><td>TN</td><td>Important</td><td>40 to 51</td><td rowspan="2">⨁⨁◯◯
LOW</td></tr>
<tr><td>FP</td><td>Important</td><td>14 to 25</td></tr>
</table></td><td>If genetic testing had been used in the reference standard, the sensitivity would likely be overestimated.</td></tr>
<tr><td>DESIRABLE EFFECTS</td><td>How substantial are the desirable anticipated effects?
o Trivial
o Small
o Moderate
o Large

o Varies
o Don't know</td><td></td><td>*The panel considered that the undesirable downstream consequences of false positive results are difficult to assess and thus uncertain for 2 main reasons: 1) false positive results could still be PCD since ongoing studies are showing that the references standards of TEM and genetic testing lack sensitivity to detect PCD (i.e. new genetic variants are discovered each year) 2) great heterogeneity in</td></tr>
</table>

UNDESIRABLE EFFECTS	**How substantial are the undesirable anticipated effects?** o Large o **Moderate** o Small o Trivial o Varies o Don't know	Imprecision and inconsistency across included studies led to decreased rating of certainty in the evidence. Detailed judgment is provided in the evidence profiles tables.	the non-PCD true underlying disease thus the expected effects of the PCD targeted pulmonary and ENT therapies. **The panel considered that the undesirable downstream consequences of false negative results difficult to assess and thus uncertain for 2 main reasons: 1) the effect could be have been underestimated since the studies assessing the impact of delayed diagnosis were not recently performed, and the standard of care has greatly improved (as well as the patient outcomes), 2) the effect could have been overestimated since older age at PCD diagnosis (usually correlated with delayed diagnosis) is associated with distrust in medical community, with less improvement in the St George's Respiratory Questionnaire scores, worsened long-term compliance with PCD treatment regimens (3) and ultimately, with worse outcomes (increased rates of respiratory cultures positive for *Pseudomonas aeruginosa* infection (4), which causes worse outcomes in similar respiratory diseases (5), increased rates of medical and surgical complications, including nasal polyposis, hemoptysis, and lobectomy surgery, all of which can cause significant morbidity and even mortality (6)).
CERTAINTY OF THE EVIDENCE OF TEST ACCURACY	**What is the overall certainty of the evidence of test accuracy?** o Very low o **Low** o Moderate	No direct evidence for critical or important direct benefits, adverse effects or burden of the test (i.e. side effects of repeat testing and anxiety related to delayed diagnosis) was considered here.	

	○ High ○ No included studies		
CERTAINTY OF THE EVIDENCE OF TEST'S EFFECTS	**What is the overall certainty of the evidence for any critical or important direct benefits, adverse effects or burden of the test?** ○ Very low ○ Low ○ Moderate ○ High **○ No included studies**	No direct evidence comparing PCD targeted pulmonary and ENT care versus no treatment was considered since these treatments consist of a bundle of different supportive therapies which are usually at least partially started for symptom relief. Nevertheless, longitudinal PCD studies show that patient using long term standard PCD regimens experienced less decline in lung function than patients left undiagnosed and thus untreated (7-9). Referral of pediatric patients to a PCD center of excellence for long-term therapies may also improve lung function and nutrition (10). Furthermore, later diagnosis (in adulthood) of PCD might be linked to worsened long-term pulmonary outcomes (7). Other individual interventions were occasionally studied but could not be pooled due to the heterogeneity of interventions and/or comparators for each critical outcome. For instance, children with PCD and chronic otitis media with effusion show marked improvements in hearing after surgical placement of ventilation tubes versus medical therapy alone (11, 12). Aggressive surgical management of chronic rhinosinusitis in PCD patients also provides significant symptom relief (13). Regular airway clearance also shows improvements in lung function in one small cross-over RCT (14).	The panel assumed that: 1) CBF analysis requires patients to travel to experienced centers, is rapid, results can be immediately available with specialized software (15), and complications of biopsy are minimal (mild discomfort, possibly mild bleeding). 2) TEM analysis often requires patients to travel to experienced centers, can take weeks to produce results, can result in non-diagnostic results requiring repeat biopsy, and complications of biopsy are minimal (mild discomfort, possibly mild bleeding) (16, 17). 3) Genetic testing does not require patient travel, can take weeks to produce results, complications of venipuncture are minimal (mild discomfort), and can result in non-diagnostic results with variants of unknown significance (18), requiring other PCD diagnostic tests. If TEM and/or genetics are replaced by CBF analysis, patients will need to travel to specialized centers for biopsy and CBF analysis. Often, travel will be required on 2-3 separate occasions, as is recommended with functional ciliary analysis, versus weeks of ciliated cell regrowth in culture with CBF analysis afterwards (19). CBF results can be

			immediately available, but the requirement of travel for repeat biopsies or weeks of cellular culture regrowth would negate this benefit.
CERTAINTY OF THE EVIDENCE OF MANAGEMENT'S EFFECTS	**What is the overall certainty of the evidence of effects of the management that is guided by the test results?** ○ Very low ○ **Low** ○ Moderate ○ High ○ No included studies	Observational studies showed that PCD patients will promptly begin standard therapies for PCD, including daily airway clearance, sputum culture surveillance, otolaryngology care, and aggressive use of antibiotics for respiratory infections (20, 21). Nevertheless, these therapies may be suboptimal outside of PCD specialized centers. Furthermore, erratic long-term compliance with PCD treatment regimens, especially in older patients at diagnosis (3), increases uncertainty regarding the link between testing and treatment.	The panel considered that standard PCD therapies are likely more efficient than what is currently reported, but equipoise would preclude studying the natural evolution of the disease without minimal intervention.
CERTAINTY OF THE EVIDENCE OF TEST RESULT/MANAGEMENT	**How certain is the link between test results and management decisions?** ○ **Very low** ○ Low ○ Moderate ○ High ○ No included studies	The overall certainty of the evidence of the effects of testing and subsequent management decisions on patient-important outcomes is limited by the very low certainty regarding the link between results and management decisions and the low certainty of the effects of the management guided by the test results.	The panel confirms that in clinical practice a positive diagnostic for PCD will almost certainly lead to chronic therapies if patient is referred to a PCD specialized center.
CERTAINTY OF EFFECTS	**What is the overall certainty of the evidence of effects of the test?**	There are also numerous publications addressing the stress created in patients surrounding their difficulty obtaining a proper PCD diagnosis. Indeed, uncertainty surrounding PCD diagnosis has been linked to poor psychosocial outcomes (22, 23). Several PCD patients and family	

	○ **Very low** ○ Low ○ Moderate ○ High ○ No included studies	representatives of PCD patients sat on this committee, and they repeatedly voiced their frustration with poor quality diagnostic testing and ambiguous diagnostic results. To these stakeholders, accurate PCD diagnosis is of the utmost importance and is the first step towards successfully managing their PCD in the long-term. Research has demonstrated that other PCD patients feel the same as our patient representatives, with many harboring distrust of the medical system over the uncertainty surrounding their PCD diagnosis. Patients also report feeling stigmatized and embarrassed due to long-term uncertainty over their PCD diagnosis (24).	
VALUES	**Is there important uncertainty about or variability in how much people value the main outcomes?** ○ Important uncertainty or variability ○ Possibly important uncertainty or variability ○ Probably no important uncertainty or variability ○ **No important uncertainty or variability**	False negative results, which are of critical importance in this analysis, are relatively more frequent with CBF testing. Patients receiving a false negative diagnosis may develop long-tern consequences by not receiving any supportive pulmonary therapies. Thus, the balance of indirect benefits/harms probably favors the reference standard.	The panel which included patients' representatives made the following assumptions about the patient-important outcomes: **Outcomes** / **Relative importance** Premature death — CRITICAL Need for lung transplant — CRITICAL Lobectomy — CRITICAL Rapid deterioration of pulmonary function — CRITICAL Restriction in physical functioning/activity — CRITICAL Development of bronchiectasies — CRITICAL Deterioration of quality of life — CRITICAL Recurrent sinopulmonary exacerbations — CRITICAL Recurrent hospitalisations — CRITICAL

			Hearing loss or speech delay — CRITICAL Recurrent antibiotics use — IMPORTANT Need for ear tube placement — IMPORTANT Need for sinus surgery — IMPORTANT Infertility — IMPORTANT Anxiety related to delayed diagnosis — IMPORTANT Side effects of repeat testing — IMPORTANT Absenteeism — IMPORTANT Poor social functioning — IMPORTANT Resources use — IMPORTANT
BALANCE OF EFFECTS	**Does the balance between desirable and undesirable effects favor the intervention or the comparison?** o Favors the comparison o **Probably favors the comparison** o Does not favor either the intervention or the comparison o Probably favors the intervention o Favors the intervention o Varies o Don't know		

	CBF (as part of HSVM)	TEM*	Genetics*
St Louis, Missouri, USA	not provided	$1,520	$950 (25)
Israel	not provided	$1,000	not provided
Southampton, UK	$1,470**	$730	not provided
Montreal, Canada	not provided	$550	$950
Denver, Colorado, USA	not provided	$715	$950
Meunster, Germany	$120**	$750	$2,900

All prices are presented in US dollars.

		*Assuming that the baseline equipment/device is already available within the hospitals offering the tests. **The purchase price of computer software required for automated CBF analysis is approximately $10,000 US dollars. CBF analysis alone is not used in any expert PCD centers. Thus, CBF calculation is part of complete functional ciliary analysis through HSVM. The cost reflects that HSVM is required to perform CBF measurement. No expert sites perform ciliary waveform analysis without high speed video recording.	
RESOURCES REQUIRED	**How large are the resource requirements (costs)?** o Large costs o Moderate costs o Negligible costs and savings o Moderate savings o Large savings o Varies o **Don't know**	All cost information was obtained from international expert PCD centers, through personal communications with center directors.	CBF measurement requires sites to purchase expensive software for automated analysis, and this analysis is usually one part of the larger HSVM, which increases costs considerably. In comparison, most academic sites already own the necessary laboratory equipment for ciliary TEM and many sites send their ciliary biopsies to third party sites for TEM processing and analysis. Genetic testing does not require institutions to purchase any start-up materials.
CERTAINTY OF EVIDENCE OF REQUIRED RESOURCES	**What is the certainty of the evidence of resource requirements (costs)?** o Very low o Low o Moderate o High	No research evidence was identified.	

	○ **No included studies**		
COST EFFECTIVENESS	**Does the cost-effectiveness of the intervention favor the intervention or the comparison?** ○ Favors the comparison ○ Probably favors the comparison ○ Does not favor either the intervention or the comparison ○ Probably favors the intervention ○ Favors the intervention ○ Varies ○ **No included studies**	No research evidence was identified.	Including the indirect costs of each PCD test above, there is not a large difference in prices for TEM, genetic, or CBF testing to diagnose PCD. Out of pocket expenses for patients are roughly equivalent for all methods, although patients do not have to pay for travel expenses with genetic testing.
EQUITY	**What would be the impact on health equity?** ○ Reduced ○ Probably reduced ○ **Probably no**	No research evidence was identified.	For patients living in remote areas, without easy access to specialized PCD centers, CBF testing would not add any convenience and would likely be infeasible. CBF calculation is a point of care test, and aside from sending a biopsied sample to a

	impact o Probably increased o Increased o Varies o Don't know		specialized PCD center for cellular regrowth in culture over many weeks, with CBF calculation afterwards, patients in remote areas could not have CBF analysis. For patients without medical insurance, the cost of a clinical visit with CBF testing is less than TEM testing, and slightly less than genetic testing, although certain companies significantly discount genetic testing for uninsured patients, making this difference small.
QU	**Is the intervention acceptable to key stakeholders?** o No o **Probably no** o Probably yes o Yes o Varies o Don't know	No research evidence was identified.	PCD stakeholders express very strong agreement with this recommendation, as they appreciate the benefits that early and accurate PCD diagnosis may have on long-term clinical and psychosocial outcomes. These PCD stakeholders strongly feel that diagnostic accuracy is of paramount importance in PCD, and accuracy outweighs all other benefits/harms of other diagnostic testing modalities, as only accurate PCD diagnosis will allow for further study of long-term PCD therapies and clinical outcomes. Stakeholders also feel it is critically important to properly diagnose PCD patients based upon genetics and/or TEM defects, in order to identify criteria causing a continued decline in this sub-group of PCD patients, which may lead to targeted, novel therapies for this sub-group.

FEASIBILITY	**Is the intervention feasible to implement?** ○ No ○ Probably no ○ **Probably yes** ○ Yes ○ Varies ○ Don't know		CBF analysis requires some practical experience and training to perform "manually" with outdated photomultiplier or photodiode techniques, and this equipment is not inexpensive to purchase and maintain. Conversely, automated CBF analysis can be accomplished through commercial software, but this must be purchased beforehand. Thus, CBF analysis is a somewhat feasible test which could be implemented in many clinical centers, following a moderate amount of setup work. Aside from purchasing of software, the hardware setup for CBF requires only standard light microscopy and recording devices, with appropriate laboratory space.

Summary of judgments – Question 4

	JUDGMENT							IMPLICATIONS
PROBLEM	No	Probably no	Probably yes	**Yes**		Varies	Don't know	
TEST ACCURACY	Very inaccurate	**Inaccurate**	Accurate	Very accurate		Varies	Don't know	
DESIRABLE EFFECTS	Trivial	**Small**	Moderate	Large		Varies	Don't know	
UNDESIRABLE EFFECTS	Large	**Moderate**	Small	Trivial		Varies	Don't know	
CERTAINTY OF THE EVIDENCE OF TEST ACCURACY	Very low	**Low**	Moderate	High			No included studies	
CERTAINTY OF THE EVIDENCE OF TEST'S EFFECTS	Very low	Low	Moderate	High			**No included studies**	

	JUDGMENT							IMPLICATIONS
CERTAINTY OF THE EVIDENCE OF MANAGEMENT'S EFFECTS	Very low	**Low**	Moderate	High			No included studies	
CERTAINTY OF THE EVIDENCE OF TEST RESULT/MANAGEMENT	Very low	**Low**	Moderate	High			No included studies	
CERTAINTY OF EFFECTS	**Very low**	Low	Moderate	High			No included studies	
VALUES	Important uncertainty or variability	Possibly important uncertainty or variability	Probably no important uncertainty or variability	**No important uncertainty or variability**				
BALANCE OF EFFECTS	Favors the comparison	**Probably favors the comparison**	Does not favor either the intervention or the comparison	Probably favors the intervention	Favors the intervention	Varies	Don't know	
RESOURCES REQUIRED	Large costs	Moderate costs	Negligible costs and savings	Moderate savings	Large savings	Varies	**Don't know**	
CERTAINTY OF EVIDENCE OF REQUIRED RESOURCES	Very low	Low	Moderate	High			**No included studies**	
COST EFFECTIVENESS	Favors the comparison	Probably favors the comparison	Does not favor either the intervention or the comparison	Probably favors the intervention	Favors the intervention	Varies	**No included studies**	
EQUITY	Reduced	Probably reduced	**Probably no impact**	Probably increased	Increased	Varies	Don't know	
ACCEPTABILITY	No	**Probably no**	Probably yes	Yes		Varies	Don't know	
FEASIBILITY	No	Probably no	**Probably yes**	Yes		Varies	Don't know	

Conclusions – Question 4

Should ciliary beat frequency (CBF) or ciliary waveform analysis using light microscopy without high speed recording, be used as a PCD diagnostic test, in adult and pediatric patients, who are at high probability of having PCD (as replacement of reference standards of classic TEM structural ciliary defect and/or biallelic causative mutations in PCD genes)?

TYPE OF RECOMMENDATION	Strong recommendation against the intervention ○	**Conditional recommendation against the intervention** ○	Conditional recommendation for either the intervention or the comparison ○	Conditional recommendation for the intervention ○	Strong recommendation for the intervention ○
RECOMMENDATION	We suggest not using CBF measurement as a diagnostic test in patients who are at high probability of having PCD. No recommendation could be made regarding the use of ciliary waveform analysis without HSVM as a diagnostic test for PCD, since no studies using currently recognized reference standards were identified by our systematic review.				
JUSTIFICATION	The overall impact of avoiding direct costs, waiting time for results, complications, and burden of repeat testing do not justify using CBF analysis as a replacement to reference standards due to the inaccuracy of CBF testing. Furthermore, CBF analysis was considered unacceptable to key stakeholders in this analysis.				
SUBGROUP CONSIDERATIONS	NA				
IMPLEMENTATION CONSIDERATIONS	We do not recommend implementation of CBF testing at this time.				
MONITORING AND EVALUATION	NA				
RESEARCH PRIORITIES	Further investigation of real-time ciliary waveform analysis without HSVM, accompanied by automated waveform and CBF interpretation software, may provide a role for real-time light microscopy analysis in the future.				

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