***A: Rana and Pokhrel Meta-analysis: Ayana 2019 (Zero\_FEC\_BaT\_K)***

# Phase 1: State the review question: Effect of inclusion of bead-beating step during DNA extraction for detection of Trichuris trichiura by qPCR

Study: : Ayana M, Cools P, Mekonnen Z, Biruksew A, Dana D, Rashwan N, et al. (2019) Comparison of

four DNA extraction and three preservation protocols for the molecular detection and quantification of soil-transmitted helminths in stool. PLoS Negl Trop Dis 13(10): e0007778.

https://doi.org/10.1371/journal.pntd.0007778

This study (Ayana et al 2019) was sub-divided into 4 sub-studies due to the nature of the study carried and couldn't be combined for analysis.

1. Ayana 2019 (Zero\_FEC\_BaT\_K)
2. Ayana 2019 (Zero\_FEC\_S\_K)
3. Ayana 2019 (Pos\_FEC\_BaT\_K)
4. Ayana 2019 (Pos\_FEC\_S\_K)

Where, Zero\_FEC means zero/negative fecal egg count (FEC) by microscopic technique, Pos\_FEC means positive (low to high intensity) fecal egg count by microscopic technique, BaT\_K means Qiagen's Blood and Tissue DNA extraction Kit, and S\_K means Qiagen's Stool DNA extraction kit.

The following analysis is for

1. Ayana 2019 (Zero\_FEC\_BaT\_K)

|  |
| --- |
| *Patients (setting, intended use of index test, presentation, prior testing): Healthy-looking school children in Jimma town, Ethiopia were enrolled. Prior testing with McMaster microscopy technique was carried for all collected stool samples, before the implementation of index and reference tests. 15 of the 174 microscopically negative samples (total n=195) were used in this sub-study. In the overall study, reference standard was combination of microscopy and/or qPCR method which consisted of DNA extraction by any kit with or without bead-beating step. But, for individual sub-studies, index test was qPCR preceded by DNA extraction by a given kit with bead-beating step during DNA extraction and reference standard was qPCR preceded by DNA extraction by same kit without the bead-beating step.* |
| *Index test(s): Index test was qPCR test preceded by DNA extraction by Qiagen's blood and tissue kit including the bead-beating step.* |
| *Reference standard and target condition: Reference test qPCR method preceded by DNA extraction by Qiagen's blood and tissue kit without bead-beating step. All healthy looking school children were target population to identify the prevalence of soil-transmitted helminths.* |

**Phase 2: Draw a flow diagram for the primary study**

1. Qiagen Stool Kit

(Zero\_FEC\_S\_K)

Include or not include

Bead-Beating step

1. Qiagen Blood and Tissue Kit

(Zero\_FEC\_BaT\_K)

Include or not include

Bead-Beating step

1. Qiagen Blood and Tissue Kit

(Zero\_FEC\_BaT\_K)

Include or not include

Bead-Beating step

1. Qiagen Stool Kit

(Zero\_FEC\_S\_K)

Include or not include

Bead-Beating step

N=195 stool samples from 5-14 years old school children from Jimma town, Ethiopia

Microscopically Positive (Positive Fecal Egg Count)

(n=21)

11 low intensity and 10 moderate-to-high intensity

Screened by McMaster Egg count method

Microscopically Negative (Zero Fecal Egg Count)

(n=174)

**Phase 3: Risk of bias and applicability judgments**

**DOMAIN 2: INDEX TEST(S)**

**If more than one index test was used, please complete for each test.**

*QUADAS-2 is structured so that 4 key domains are each rated in terms of the risk of bias and* the concern regarding applicability to the research question (as defined above). Each key domain has a set of signalling questions to help reach the judgments regarding bias and applicability.

|  |  |  |
| --- | --- | --- |
| **DOMAIN 1: PATIENT SELECTION**  **A. Risk of Bias** |  |  |
| Describe methods of patient selection: All healthy school student providing enough stools and informed consent were enrolled in the study. Few of the microscopically negative stool samples were chosen for analysis. While consecutive samples were taken, this sub-study used a sub-set of microscopically negative samples. | | |
| * Was a consecutive or random sample of patients enrolled? | | Unclear |
| * Was a case-control design avoided? |  | Yes |
| * Did the study avoid inappropriate exclusions? |  | Unclear |
| **Could the selection of patients have introduced bias?** | **RISK: UNCLEAR** | |
| **B. Concerns regarding applicability** |  |  |
| Describe included patients (prior testing, presentation, intended use of index test and setting)**:** Health school children were enrolled. Stool samples were microscopically tested (McMaster technique) before using the qPCR. Both reference and index tests were used simultaneously**.** | | |
| **Is there concern that the included patients do not match the review question?** | **CONCERN: LOW** | |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **A. Risk of Bias** |  |  |
| Describe the index test and how it was conducted and interpreted: Index test was qPCR preceded by DNA extraction by Qiagen blood and tissue kit including bead-beating step.Out of all collected samples, only a sub-set of microscopically negative samples were used in the test.  Threshold is also called analytical sensitivity for qPCR. As identical qPCR is used for both index and reference test, this will not impact data interpretation. Index and reference test differ only in DNA extraction stage. | | |  |
|  | * Were the index test results interpreted without   knowledge of the results of the reference standard? | Yes |  |
|  | * If a threshold was used, was it pre-specified? | Yes |  |
|  | **Could the conduct or interpretation of the index test**  **have introduced bias?** | **RISK: LOW** |  |
|  | **B. Concerns regarding applicability** |  |  |
|  | **Is there concern that the index test, its conduct, or interpretation differ from the review question?** | **CONCERN: LOW** |  |

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| --- | --- | --- |
| **DOMAIN 3: REFERENCE STANDARD**  **A. Risk of Bias** |  |  |
| Describe the reference standard and how it was conducted and interpreted:  Reference standard consisted of the same qPCR as index but preceded by the DNA extraction kit without the bead-beating step. The test samples were aliquoted into two, and were tested by Index test and Reference standard separately. qPCR has very high sensitivity and specificity and is method of choice. | | |
| * Is the reference standard likely to correctly classify the target   condition? | | Yes |
| * Were the reference standard results interpreted without knowledge of the results of the index test? | | Yes |
| **Could the reference standard, its conduct, or its**  **interpretation have introduced bias?** | **RISK: LOW** | |
| **B. Concerns regarding applicability** |  |  |
| **Is there concern that the target condition as defined by the reference standard does not match the review question?** | **CONCERN: LOW** | |

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| --- | --- | --- |
| **DOMAIN 4: FLOW AND TIMING**  **A. Risk of Bias** |  |  |
| Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2x2 table (refer to flow diagram):  *All of the samples were aliquoted and tested by both index test and reference standard.*  Describe the time interval and any interventions between index test(s) and reference standard:  The time difference was zero. As aliquots of same sample was tested by both method. | | |
| * Was there an appropriate interval between index test(s)   and reference standard? | | Yes |
| * Did all patients receive a reference standard? |  | Yes |
| * Did patients receive the same reference standard? |  | Yes |
| * Were all patients included in the analysis? |  | Yes |
| **Could the patient flow have introduced bias?** | **RISK: LOW** | |

***B: Rana and Pokhrel Meta-analysis: Ayana 2019 (Zero\_FEC\_S\_K)***

# Phase 1: State the review question: Effect of inclusion of bead-beating step during DNA extraction for detection of Trichuris trichiura by qPCR

Study: : Ayana M, Cools P, Mekonnen Z, Biruksew A, Dana D, Rashwan N, et al. (2019) Comparison of

four DNA extraction and three preservation protocols for the molecular detection and quantification of soil-transmitted helminths in stool. PLoS Negl Trop Dis 13(10): e0007778.

https://doi.org/10.1371/journal.pntd.0007778

This study (Ayana et al 2019) was sub-divided into 4 sub-studies due to the nature of the study carried and couldn't be combined for analysis.

1. Ayana 2019 (Zero\_FEC\_BaT\_K)
2. Ayana 2019 (Zero\_FEC\_S\_K)
3. Ayana 2019 (Pos\_FEC\_BaT\_K)
4. Ayana 2019 (Pos\_FEC\_S\_K)

Where, Zero\_FEC means zero/negative fecal egg count (FEC) by microscopic technique, Pos\_FEC means positive (low to high intensity) fecal egg count by microscopic technique, BaT\_K means Qiagen's Blood and Tissue DNA extraction Kit, and S\_K means Qiagen's Stool DNA extraction kit.

The following analysis is for

1. Ayana 2019 (Zero\_FEC\_S\_K)

|  |
| --- |
| *Patients (setting, intended use of index test, presentation, prior testing): Healthy-looking school children in Jimma town, Ethiopia were enrolled. Prior testing with McMaster microscopy technique was carried for all collected stool samples, before the implementation of index and reference tests. 15 of the 174 microscopically negative samples (total n=195) were used in this sub-study. In the overall study, reference standard was combination of microscopy and/or qPCR method which consisted of DNA extraction by any kit with or without bead-beating step. But, for individual sub-studies, index test was qPCR preceded by DNA extraction by a given kit with bead-beating step during DNA extraction and reference standard was qPCR preceded by DNA extraction by same kit without the bead-beating step.* |
| *Index test(s): Index test was qPCR test preceded by DNA extraction by Qiagen's stool SNA extraction kit including the bead-beating step.* |
| *Reference standard and target condition: Reference test qPCR method preceded by DNA extraction by Qiagen's stool DNA extraction kit without bead-beating step. All healthy looking school children were target population to identify the prevalence of soil-transmitted helminths.* |

**Phase 2: Draw a flow diagram for the primary study**

1. Qiagen Blood and Tissue Kit

(Zero\_FEC\_BaT\_K)

Include or not include

Bead-Beating step

1. Qiagen Stool Kit

(Zero\_FEC\_S\_K)

Include or not include

Bead-Beating step

1. Qiagen Blood and Tissue Kit

(Zero\_FEC\_BaT\_K)

Include or not include

Bead-Beating step

1. Qiagen Stool Kit

(Zero\_FEC\_S\_K)

Include or not include

Bead-Beating step

N=195 stool samples from 5-14 years old school children from Jimma town, Ethiopia

Microscopically Positive (Positive Fecal Egg Count)

(n=21)

11 low intensity and 10 moderate-to-high intensity

Screened by McMaster Egg count method

Microscopically Negative (Zero Fecal Egg Count)

(n=174)

**Phase 3: Risk of bias and applicability judgments**

**DOMAIN 2: INDEX TEST(S)**

**If more than one index test was used, please complete for each test.**

*QUADAS-2 is structured so that 4 key domains are each rated in terms of the risk of bias and* the concern regarding applicability to the research question (as defined above). Each key domain has a set of signalling questions to help reach the judgments regarding bias and applicability.

|  |  |  |
| --- | --- | --- |
| **DOMAIN 1: PATIENT SELECTION**  **A. Risk of Bias** |  |  |
| Describe methods of patient selection: All healthy school student providing enough stools and informed consent were enrolled in the study. Few of the microscopically negative stool samples were chosen for analysis. While consecutive samples were taken, this sub-study used a sub-set of microscopically negative samples. | | |
| * Was a consecutive or random sample of patients enrolled? | | Unclear |
| * Was a case-control design avoided? |  | Yes |
| * Did the study avoid inappropriate exclusions? |  | Unclear |
| **Could the selection of patients have introduced bias?** | **RISK: UNCLEAR** | |
| **B. Concerns regarding applicability** |  |  |
| Describe included patients (prior testing, presentation, intended use of index test and setting)**:** Health school children were enrolled. Stool samples were microscopically tested (McMaster technique) before using the qPCR. Both reference and index tests were used simultaneously**.** | | |
| **Is there concern that the included patients do not match the review question?** | **CONCERN: LOW** | |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **A. Risk of Bias** |  |  |
| Describe the index test and how it was conducted and interpreted: Index test was qPCR preceded by DNA extraction by Qiagen stool DNA extraction kit including bead-beating step.Out of all collected samples, only a sub-set of microscopically negative samples were used in the test.  Threshold is also called analytical sensitivity for qPCR. As identical qPCR is used for both index and reference test, this will not impact data interpretation. Index and reference test differ only in DNA extraction stage. | | |  |
|  | * Were the index test results interpreted without   knowledge of the results of the reference standard? | Yes |  |
|  | * If a threshold was used, was it pre-specified? | Yes |  |
|  | **Could the conduct or interpretation of the index test**  **have introduced bias?** | **RISK: LOW** |  |
|  | **B. Concerns regarding applicability** |  |  |
|  | **Is there concern that the index test, its conduct, or interpretation differ from the review question?** | **CONCERN: LOW** |  |

|  |  |  |
| --- | --- | --- |
| **DOMAIN 3: REFERENCE STANDARD**  **A. Risk of Bias** |  |  |
| Describe the reference standard and how it was conducted and interpreted:  Reference standard consisted of the same qPCR but preceded by the DNA extraction kit without the bead-beating step. The test samples were aliquoted into two,and were tested by Index test and Reference standard. qPCR has very high sensitivity and specificity and is method of choice. | | |
| * Is the reference standard likely to correctly classify the target   condition? | | Yes |
| * Were the reference standard results interpreted without knowledge of the results of the index test? | | Yes |
| **Could the reference standard, its conduct, or its**  **interpretation have introduced bias?** | **RISK: LOW** | |
| **B. Concerns regarding applicability** |  |  |
| **Is there concern that the target condition as defined by the reference standard does not match the review question?** | **CONCERN: LOW** | |

|  |  |  |
| --- | --- | --- |
| **DOMAIN 4: FLOW AND TIMING**  **A. Risk of Bias** |  |  |
| Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2x2 table (refer to flow diagram):  *All of the samples were aliquoted and tested by both index test and reference standard.*  Describe the time interval and any interventions between index test(s) and reference standard:  The time difference was zero. As aliquots of same sample was tested by both method. | | |
| * Was there an appropriate interval between index test(s)   and reference standard? | | Yes |
| * Did all patients receive a reference standard? |  | Yes |
| * Did patients receive the same reference standard? |  | Yes |
| * Were all patients included in the analysis? |  | Yes |
| **Could the patient flow have introduced bias?** | **RISK: LOW** | |

***C: Rana and Pokhrel Meta-analysis: Ayana 2019 (Pos\_FEC\_BaT\_K)***

# Phase 1: State the review question: Effect of inclusion of bead-beating step during DNA extraction for detection of Trichuris trichiura by qPCR

Study: : Ayana M, Cools P, Mekonnen Z, Biruksew A, Dana D, Rashwan N, et al. (2019) Comparison of

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2. Ayana 2019 (Zero\_FEC\_S\_K)
3. Ayana 2019 (Pos\_FEC\_BaT\_K)
4. Ayana 2019 (Pos\_FEC\_S\_K)

Where, Zero\_FEC means zero/negative fecal egg count (FEC) by microscopic technique, Pos\_FEC means positive (low to high intensity) fecal egg count by microscopic technique, BaT\_K means Qiagen's Blood and Tissue DNA extraction Kit, and S\_K means Qiagen's Stool DNA extraction kit.

The following analysis is for

1. Ayana 2019 (Pos\_FEC\_BaT\_K)

|  |
| --- |
| *Patients (setting, intended use of index test, presentation, prior testing): Healthy-looking school children in Jimma town, Ethiopia were enrolled. Prior testing with McMaster microscopy technique was carried for all collected stool samples, before the implementation of index and reference tests. The author of this systematic review/meta-analysis combined low intensity (n=11) and moderate-to-heavy intensity (n=10) samples into one group as positive. 21 microscopically positive samples (total n=195) were used in this sub-study. In the overall study, reference standard was combination of microscopy and/or qPCR method which consisted of DNA extraction by any kit with or without bead-beating step. But, for individual sub-studies, index test was qPCR preceded by DNA extraction by a given kit with bead-beating step during DNA extraction and reference standard was qPCR preceded by DNA extraction by same kit without the bead-beating step.* |
| *Index test(s): Index test was qPCR test preceded by DNA extraction by Qiagen's blood and tissue kit including the bead-beating step.* |
| *Reference standard and target condition: Reference test qPCR method preceded by DNA extraction by Qiagen's blood and tissue kit without bead-beating step. All healthy looking school children were target population to identify the prevalence of soil-transmitted helminths.* |

**Phase 2: Draw a flow diagram for the primary study**

1. Qiagen Stool Kit

(Zero\_FEC\_S\_K)

Include or not include

Bead-Beating step

1. Qiagen Blood and Tissue Kit

(Zero\_FEC\_BaT\_K)

Include or not include

Bead-Beating step

1. Qiagen Blood and Tissue Kit

(Zero\_FEC\_BaT\_K)

Include or not include

Bead-Beating step

1. Qiagen Stool Kit

(Zero\_FEC\_S\_K)

Include or not include

Bead-Beating step

N=195 stool samples from 5-14 years old school children from Jimma town, Ethiopia

Microscopically Positive (Positive Fecal Egg Count)

(n=21)

11 low intensity and 10 moderate-to-high intensity

Screened by McMaster Egg count method

Microscopically Negative (Zero Fecal Egg Count)

(n=174)

**Phase 3: Risk of bias and applicability judgments**

**DOMAIN 2: INDEX TEST(S)**

**If more than one index test was used, please complete for each test.**

*QUADAS-2 is structured so that 4 key domains are each rated in terms of the risk of bias and* the concern regarding applicability to the research question (as defined above). Each key domain has a set of signalling questions to help reach the judgments regarding bias and applicability.

|  |  |  |
| --- | --- | --- |
| **DOMAIN 1: PATIENT SELECTION**  **A. Risk of Bias** |  |  |
| Describe methods of patient selection: All healthy school student providing enough stools and informed consent were enrolled in the study. All of the 21 microscopically positive stool samples were chosen for analysis. The whole sample (n=195) were taken consecutively, thus the positive samples can be considered consecutive. | | |
| * Was a consecutive or random sample of patients enrolled? | | Yes |
| * Was a case-control design avoided? |  | Yes |
| * Did the study avoid inappropriate exclusions? |  | Yes |
| **Could the selection of patients have introduced bias?** | **RISK: LOW** | |
| **B. Concerns regarding applicability** |  |  |
| Describe included patients (prior testing, presentation, intended use of index test and setting)**:** Health school children were enrolled. Stool samples were microscopically tested (McMaster technique) before using the qPCR. Both reference and index tests were used simultaneously**.** | | |
| **Is there concern that the included patients do not match the review question?** | **CONCERN: LOW** | |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **A. Risk of Bias** |  |  |
| Describe the index test and how it was conducted and interpreted: Index test was qPCR preceded by DNA extraction by Qiagen Blood and tissue kit including bead-beating step.Out of all collected samples, all of the microscopically positive samples were used in the test.  Threshold is also called analytical sensitivity for qPCR. As identical qPCR is used for both index and reference test, this will not impact data interpretation. Index and reference test differ only in DNA extraction stage. | | |  |
|  | * Were the index test results interpreted without   knowledge of the results of the reference standard? | Yes |  |
|  | * If a threshold was used, was it pre-specified? | Yes |  |
|  | **Could the conduct or interpretation of the index test**  **have introduced bias?** | **RISK: LOW** |  |
|  | **B. Concerns regarding applicability** |  |  |
|  | **Is there concern that the index test, its conduct, or interpretation differ from the review question?** | **CONCERN: LOW** |  |

|  |  |  |
| --- | --- | --- |
| **DOMAIN 3: REFERENCE STANDARD**  **A. Risk of Bias** |  |  |
| Describe the reference standard and how it was conducted and interpreted:  Reference standard consisted of the same qPCR but preceded by the DNA extraction kit without the bead-beating step. The test samples were aliquoted into two,and were tested by Index test and Reference standard. qPCR has very high sensitivity and specificity and is method of choice. | | |
| * Is the reference standard likely to correctly classify the target   condition? | | Yes |
| * Were the reference standard results interpreted without knowledge of the results of the index test? | | Yes |
| **Could the reference standard, its conduct, or its**  **interpretation have introduced bias?** | **RISK: LOW** | |
| **B. Concerns regarding applicability** |  |  |
| **Is there concern that the target condition as defined by the reference standard does not match the review question?** | **CONCERN: LOW** | |

|  |  |  |
| --- | --- | --- |
| **DOMAIN 4: FLOW AND TIMING**  **A. Risk of Bias** |  |  |
| Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2x2 table (refer to flow diagram):  *All of the samples were aliquoted and tested by both index test and reference standard.*  Describe the time interval and any interventions between index test(s) and reference standard:  The time difference was zero. As aliquots of same sample was tested by both method. | | |
| * Was there an appropriate interval between index test(s)   and reference standard? | | Yes |
| * Did all patients receive a reference standard? |  | Yes |
| * Did patients receive the same reference standard? |  | Yes |
| * Were all patients included in the analysis? |  | Yes |
| **Could the patient flow have introduced bias?** | **RISK: LOW** | |

***D: Rana and Pokhrel Meta-analysis: Ayana 2019 (Pos\_FEC\_S\_K)***

# Phase 1: State the review question: Effect of inclusion of bead-beating step during DNA extraction for detection of Trichuris trichiura by qPCR

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Where, Zero\_FEC means zero/negative fecal egg count (FEC) by microscopic technique, Pos\_FEC means positive (low to high intensity) fecal egg count by microscopic technique, BaT\_K means Qiagen's Blood and Tissue DNA extraction Kit, and S\_K means Qiagen's Stool DNA extraction kit.

The following analysis is for

4 Ayana 2019 (Pos\_FEC\_S\_K)

|  |
| --- |
| *Patients (setting, intended use of index test, presentation, prior testing): Healthy-looking school children in Jimma town, Ethiopia were enrolled. Prior testing with McMaster microscopy technique was carried for all collected stool samples, before the implementation of index and reference tests. The author of this systematic review/meta-analysis combined low intensity (n=11) and moderate-to-heavy intensity (n=10) samples into one group as positive. 21 microscopically positive samples (total n=195) were used in this sub-study. In the overall study, reference standard was combination of microscopy and/or qPCR method which consisted of DNA extraction by any kit with or without bead-beating step. But, for individual sub-studies, index test was qPCR preceded by DNA extraction by a given kit with bead-beating step during DNA extraction and reference standard was qPCR preceded by DNA extraction by same kit without the bead-beating step.* |
| *Index test(s): Index test was qPCR test preceded by DNA extraction by Qiagen's stool DNA extraction kit including the bead-beating step.* |
| *Reference standard and target condition: Reference test qPCR method preceded by DNA extraction by Qiagen's stool DNA extraction kit without bead-beating step. All healthy looking school children were target population to identify the prevalence of soil-transmitted helminths.* |

**Phase 2: Draw a flow diagram for the primary study**

1. Qiagen Stool Kit

(Zero\_FEC\_S\_K)

Include or not include

Bead-Beating step

1. Qiagen Blood and Tissue Kit

(Zero\_FEC\_BaT\_K)

Include or not include

Bead-Beating step

1. Qiagen Blood and Tissue Kit

(Zero\_FEC\_BaT\_K)

Include or not include

Bead-Beating step

1. Qiagen Stool Kit

(Zero\_FEC\_S\_K)

Include or not include

Bead-Beating step

N=195 stool samples from 5-14 years old school children from Jimma town, Ethiopia

Microscopically Positive (Positive Fecal Egg Count)

(n=21)

11 low intensity and 10 moderate-to-high intensity

Screened by McMaster Egg count method

Microscopically Negative (Zero Fecal Egg Count)

(n=174)

**Phase 3: Risk of bias and applicability judgments**

**DOMAIN 2: INDEX TEST(S)**

**If more than one index test was used, please complete for each test.**

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|  |  |  |
| --- | --- | --- |
| **DOMAIN 1: PATIENT SELECTION**  **A. Risk of Bias** |  |  |
| Describe methods of patient selection: All healthy school student providing enough stools and informed consent were enrolled in the study. All of the 21 microscopically positive stool samples were chosen for analysis. The whole sample (n=195) were taken consecutively, thus the positive samples can be considered consecutive. | | |
| * Was a consecutive or random sample of patients enrolled? | | Yes |
| * Was a case-control design avoided? |  | Yes |
| * Did the study avoid inappropriate exclusions? |  | Yes |
| **Could the selection of patients have introduced bias?** | **RISK: LOW** | |
| **B. Concerns regarding applicability** |  |  |
| Describe included patients (prior testing, presentation, intended use of index test and setting)**:** Health school children were enrolled. Stool samples were microscopically tested (McMaster technique) before using the qPCR. Both reference and index tests were used simultaneously**.** | | |
| **Is there concern that the included patients do not match the review question?** | **CONCERN: LOW** | |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **A. Risk of Bias** |  |  |
| Describe the index test and how it was conducted and interpreted: Index test was qPCR preceded by DNA extraction by Qiagen stool DNA extraction kit including bead-beating step.Out of all collected samples, all of the microscopically positive samples were used in the test.  Threshold is also called analytical sensitivity for qPCR. As identical qPCR is used for both index and reference test, this will not impact data interpretation. Index and reference test differ only in DNA extraction stage. | | |  |
|  | * Were the index test results interpreted without   knowledge of the results of the reference standard? | Yes |  |
|  | * If a threshold was used, was it pre-specified? | Yes |  |
|  | **Could the conduct or interpretation of the index test**  **have introduced bias?** | **RISK: LOW** |  |
|  | **B. Concerns regarding applicability** |  |  |
|  | **Is there concern that the index test, its conduct, or interpretation differ from the review question?** | **CONCERN: LOW** |  |

|  |  |  |
| --- | --- | --- |
| **DOMAIN 3: REFERENCE STANDARD**  **A. Risk of Bias** |  |  |
| Describe the reference standard and how it was conducted and interpreted:  Reference standard consisted of the same qPCR but preceded by the DNA extraction kit without the bead-beating step. The test samples were aliquoted into two,and were tested by Index test and Reference standard. qPCR has very high sensitivity and specificity and is method of choice. | | |
| * Is the reference standard likely to correctly classify the target   condition? | | Yes |
| * Were the reference standard results interpreted without knowledge of the results of the index test? | | Yes |
| **Could the reference standard, its conduct, or its**  **interpretation have introduced bias?** | **RISK: LOW** | |
| **B. Concerns regarding applicability** |  |  |
| **Is there concern that the target condition as defined by the reference standard does not match the review question?** | **CONCERN: LOW** | |

|  |  |  |
| --- | --- | --- |
| **DOMAIN 4: FLOW AND TIMING**  **A. Risk of Bias** |  |  |
| Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2x2 table (refer to flow diagram):  *All of the samples were aliquoted and tested by both index test and reference standard.*  Describe the time interval and any interventions between index test(s) and reference standard:  The time difference was zero. As aliquots of same sample was tested by both method. | | |
| * Was there an appropriate interval between index test(s)   and reference standard? | | Yes |
| * Did all patients receive a reference standard? |  | Yes |
| * Did patients receive the same reference standard? |  | Yes |
| * Were all patients included in the analysis? |  | Yes |
| **Could the patient flow have introduced bias?** | **RISK: LOW** | |

***E: Rana and Pokhrel Meta-analysis: Kaisar 2017 (Frozen\_samp)***

**Phase 1: State the review question: Effect of inclusion of bead-beating step during DNA extraction for detection of Trichuris trichiura by qPCR**

Study: Kaisar MM, Brienen EA, Djuardi Y, Sartono E, Yazdanbakhsh M, Verweij JJ, Supali T, Van Lieshout L. Improved diagnosis of Trichuris trichiura by using a bead-beating procedure on ethanol preserved stool samples prior to DNA isolation and the performance of multiplex real-time PCR for intestinal parasites. Parasitology. 2017 Jun;144(7):965-74.

This study (Kaisar et al 2017) two set of experiments. Each of the samples (n=60) were aliquoted into two, one frozen for preservation and the other mixed with ethanol and preserved at room temperature. Each aliquot was further aliquoted into two and DNA was extracted with either incorporation of bead-beating step during extraction or without the step. As the preservation technique was different in the two experiments, we have divided the study (Kaisar et al 2017) into two sub-studies:

1. Kaisar 2017 (Frozen\_samp), and
2. Kaisar 2017 (Ethanol\_samp)

This analysis is for

1. Kaisar 2017 (Frozen\_samp)

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| *Patients (setting, intended use of index test, presentation, prior testing):* Total of 60 samples were collected from 38 mothers (20-37 years, median 34 years) and 22 children (1-5 years, median 3 years) living in Nangapanda sub-district, Ende, Flores Island, Indonesia. Patient population were suspected to be infected with soil-transmitted helminth to some degree but were apparently healthy. All samples were tested with Kato-Katz microscopy method before aliquotation. Each of the samples were aliquoted and either frozen at -20C or mixed with ethanol and preserved at room temperature. Frozen samples were analyzed in this study. |
| *Index test(s): The frozen samples were tested by qPCR preceded by DNA extraction containing bead-beating step.* |
| *Reference standard and target condition: Reference standard also contained qPCR testing preceded by DNA extraction without the bead-beating step. Frozen samples were processed. Patients (mothers and their children) were chosen based on suspicion of helminth infection and were apparently healthy.* |

**Phase 2: Draw a flow diagram for the primary study**

Preserved by mixing with ethanol

>sub-aliquoted and processed by DNA extraction with (index) or without (reference) bead-beating

Preserved by freezing

>sub-aliquoted and processed by DNA extraction with (index) or without (reference) bead-beating

Aliquoted into two

All samples tested by Kato-Katz microscopy technique

N=60 stool samples consecutively collected from 38 mothers and their 22 children from living in Nangapanda sub-district, Ende, Flores Island, Indonesia.

**Phase 3: Risk of bias and applicability judgments**

**DOMAIN 2: INDEX TEST(S)**

**If more than one index test was used, please complete for each test.**

*QUADAS-2 is structured so that 4 key domains are each rated in terms of the risk of bias and* the concern regarding applicability to the research question (as defined above). Each key domain has a set of signalling questions to help reach the judgments regarding bias and applicability.

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| **DOMAIN 1: PATIENT SELECTION**  **A. Risk of Bias** |  |  |
| Describe methods of patient selection: Consecutive patients were selected. First 60 samples were used in the project. | | |
| * Was a consecutive or random sample of patients enrolled? | | Yes |
| * Was a case-control design avoided? |  | Yes |
| * Did the study avoid inappropriate exclusions? |  | Yes |
| **Could the selection of patients have introduced bias?** | **RISK: LOW** | |
| **B. Concerns regarding applicability** |  |  |
| Describe included patients (prior testing, presentation, intended use of index test and setting)**:**  While all samples are tested by a low-sensitivity microscopy technique, Kato-Katz, this doesnt impact the implementation of index and reference tests which are done simultaneously for all samples. | | |
| **Is there concern that the included patients do not match the review question?** | **CONCERN: LOW** | |

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|  | **A. Risk of Bias** |  |  |
| Describe the index test and how it was conducted and interpreted: Suspected population (infected with helminths) is chosen based on earlier studies. No specific symptoms or clinical characteristics are chosen for enrollment. All people given consent are enrolled and first 60 samples are enrolled. Kato-Katz is carried for all samples and 2 aliquots of each sample are frozen or preserved by mixing with ethanol. Index test is qPCR precededby DNA extraction using bead-beating step. The index test was interpreted without the knowledge of reference test.  Threshold is also called analytical sensitivity for qPCR. As identical qPCR is used for both index and reference test, this will not impact data interpretation. Index and reference test differ only in DNA extraction stage. | | |  |
|  | * Were the index test results interpreted without   knowledge of the results of the reference standard? | Yes |  |
|  | * If a threshold was used, was it pre-specified? | Yes |  |
|  | **Could the conduct or interpretation of the index test**  **have introduced bias?** | **RISK: LOW** |  |
|  | **B. Concerns regarding applicability** |  |  |
|  | **Is there concern that the index test, its conduct, or interpretation differ from the review question?** | **CONCERN: LOW** |  |

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| **DOMAIN 3: REFERENCE STANDARD**  **A. Risk of Bias** |  |  |
| Describe the reference standard and how it was conducted and interpreted:  While the overall reference to calculate the prevalence of soil-transmitted helminth in the target population was combination of microscopy and qPCR irrespective of methodological differences, the reference test to analyze the review question was qPCR with normal DNA extraction without bead beating step. | | |
| * Is the reference standard likely to correctly classify the target   condition? | | Yes |
| * Were the reference standard results interpreted without knowledge of the results of the index test? | | Yes |
| **Could the reference standard, its conduct, or its**  **interpretation have introduced bias?** | **RISK: LOW** | |
| **B. Concerns regarding applicability** |  |  |
| **Is there concern that the target condition as defined by the reference standard does not match the review question?** | **CONCERN: LOW** | |

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| **DOMAIN 4: FLOW AND TIMING**  **A. Risk of Bias** |  |  |
| Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2x2 table (refer to flow diagram):  All of the samples were aliquoted and done by both index and reference tests.  Describe the time interval and any interventions between index test(s) and reference standard:  As same samples were divided and aliquoted for index and reference tests, there was no difference in time. Further, processing were done on together. | | |
| * Was there an appropriate interval between index test(s)   and reference standard? | | Yes |
| * Did all patients receive a reference standard? |  | Yes |
| * Did patients receive the same reference standard? |  | Yes |
| * Were all patients included in the analysis? |  | Yes |
| **Could the patient flow have introduced bias?** | **RISK: LOW** | |

***F: Rana and Pokhrel Meta-analysis: Kaisar 2017 (Ethanol\_samp)***

**Phase 1: State the review question: Effect of inclusion of bead-beating step during DNA extraction for detection of Trichuris trichiura by qPCR**

Study: Kaisar MM, Brienen EA, Djuardi Y, Sartono E, Yazdanbakhsh M, Verweij JJ, Supali T, Van Lieshout L. Improved diagnosis of Trichuris trichiura by using a bead-beating procedure on ethanol preserved stool samples prior to DNA isolation and the performance of multiplex real-time PCR for intestinal parasites. Parasitology. 2017 Jun;144(7):965-74.

This study (Kaisar et al 2017) two set of experiments. Each of the samples (n=60) were aliquoted into two, one frozen for preservation and the other mixed with ethanol and preserved at room temperature. Each aliquot was further aliquoted into two and DNA was extracted with either incorporation of bead-beating step during extraction or without the step. As the preservation technique was different in the two experiments, we have divided the study (Kaisar et al 2017) into two sub-studies:

1. Kaisar 2017 (Frozen\_samp), and
2. Kaisar 2017 (Ethanol\_samp)

This analysis is for

1. Kaisar 2017 (Ethanol\_samp)

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| --- |
| *Patients (setting, intended use of index test, presentation, prior testing):* Total of 60 samples were collected from 38 mothers (20-37 years, median 34 years) and 22 children (1-5 years, median 3 years) living in Nangapanda sub-district, Ende, Flores Island, Indonesia. Patient population were suspected to be infected with soil-transmitted helminth to some degree but were apparently healthy. All samples were tested with Kato-Katz microscopy method before aliquotation. Each of the samples were aliquoted and either frozen at -20C or mixed with ethanol and preserved at room temperature. In this sub-study, ethanol preserved samples were processed. |
| *Index test(s): The ethanol preserved samples were tested by qPCR preceded by DNA extraction containing bead-beating step.* |
| *Reference standard and target condition: Reference standard also contained qPCR testing preceded by DNA extraction without the bead-beating step. Ethanol preserved samples were tested. Patients (mothers and their children) were chosen based on suspicion of helminth infection and were apparently healthy.* |

**Phase 2: Draw a flow diagram for the primary study**

Preserved by mixing with ethanol

>sub-aliquoted and processed by DNA extraction with (index) or without (reference) bead-beating

Preserved by freezing

>sub-aliquoted and processed by DNA extraction with (index) or without (reference) bead-beating

Aliquoted into two

All samples tested by Kato-Katz microscopy technique

N=60 stool samples consecutively collected from 38 mothers and their 22 children from living in Nangapanda sub-district, Ende, Flores Island, Indonesia.

**Phase 3: Risk of bias and applicability judgments**

**DOMAIN 2: INDEX TEST(S)**

**If more than one index test was used, please complete for each test.**

*QUADAS-2 is structured so that 4 key domains are each rated in terms of the risk of bias and* the concern regarding applicability to the research question (as defined above). Each key domain has a set of signalling questions to help reach the judgments regarding bias and applicability.

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| **DOMAIN 1: PATIENT SELECTION**  **A. Risk of Bias** |  |  |
| Describe methods of patient selection: Consecutive patients were selected. First 60 samples were used in the project. | | |
| * Was a consecutive or random sample of patients enrolled? | | Yes |
| * Was a case-control design avoided? |  | Yes |
| * Did the study avoid inappropriate exclusions? |  | Yes |
| **Could the selection of patients have introduced bias?** | **RISK: LOW** | |
| **B. Concerns regarding applicability** |  |  |
| Describe included patients (prior testing, presentation, intended use of index test and setting)**:**  While all samples are tested by a low-sensitivity microscopy technique, Kato-Katz, this doesnt impact the implementation of index and reference tests which are done simultaneously for all samples. | | |
| **Is there concern that the included patients do not match the review question?** | **CONCERN: LOW** | |

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|  | **A. Risk of Bias** |  |  |
| Describe the index test and how it was conducted and interpreted: Suspected population (infected with helminths) is chosen based on earlier studies. No specific symptoms or clinical characteristics are chosen for enrollment. All people given consent are enrolled and first 60 samples are enrolled. Kato-Katz is carried for all samples and 2 aliquots of each sample are frozen or preserved by mixing with ethanol. Index test is qPCR preceded by DNA extraction using bead-beating step. The index test was interpreted without the knowledge of reference test.  Threshold is also called analytical sensitivity for qPCR. As identical qPCR is used for both index and reference test, this will not impact data interpretation. Index and reference test differ only in DNA extraction stage. | | |  |
|  | * Were the index test results interpreted without   knowledge of the results of the reference standard? | Yes |  |
|  | * If a threshold was used, was it pre-specified? | Yes |  |
|  | **Could the conduct or interpretation of the index test**  **have introduced bias?** | **RISK: LOW** |  |
|  | **B. Concerns regarding applicability** |  |  |
|  | **Is there concern that the index test, its conduct, or interpretation differ from the review question?** | **CONCERN: LOW** |  |

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| **DOMAIN 3: REFERENCE STANDARD**  **A. Risk of Bias** |  |  |
| Describe the reference standard and how it was conducted and interpreted:  While the overall reference to calculate the prevalence of soil-transmitted helminth in the target population was combination of microscopy and qPCR irrespective of methodological differences, the reference test to analyze the review question was qPCR with normal DNA extraction without bead beating step. | | |
| * Is the reference standard likely to correctly classify the target   condition? | | Yes |
| * Were the reference standard results interpreted without knowledge of the results of the index test? | | Yes |
| **Could the reference standard, its conduct, or its**  **interpretation have introduced bias?** | **RISK: LOW** | |
| **B. Concerns regarding applicability** |  |  |
| **Is there concern that the target condition as defined by the reference standard does not match the review question?** | **CONCERN: LOW** | |

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| **DOMAIN 4: FLOW AND TIMING**  **A. Risk of Bias** |  |  |
| Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2x2 table (refer to flow diagram):  All of the samples were aliquoted and done by both index and reference tests.  Describe the time interval and any interventions between index test(s) and reference standard:  As same samples were divided and aliquoted for index and reference tests, there was no difference in time. Further, processing were done on together. | | |
| * Was there an appropriate interval between index test(s)   and reference standard? | | Yes |
| * Did all patients receive a reference standard? |  | Yes |
| * Did patients receive the same reference standard? |  | Yes |
| * Were all patients included in the analysis? |  | Yes |
| **Could the patient flow have introduced bias?** | **RISK: LOW** | |