

Standard Method Performance Requirements for Immunological-Based Handheld Assays (HHAs) for Detection of Ricin in Visible Powders

Intended Use: Field use by first responders for analysis of visible powders

Method Developer and Independent Validation Studies

Probability of Detection at the Acceptable Minimum Detection Level

1 Definitions

Probability of detection (POD) is the proportion of positive analytical outcomes for a qualitative method for a given matrix at a given agent level or concentration. POD is concentration-dependent. The acceptable minimum detection level (AMDL) is the predetermined minimum level of a biological threat agent, which must be detected by the candidate method with an estimated 5% lower confidence limit on the POD of 0.95 or higher. The AMDL is dependent on the intended use.

2 Test Conditions

AMDL is 25 ng/mL *Ricinus Communis* Agglutinin II (RCA 60) in candidate method sample collection buffer.

3 Acceptance Criteria

Estimated 5% lower confidence limit on the POD must be 0.95 or higher. (No more than one failure in 96 replicates.)

Inclusivity

1 Definition

Strains or isolates or variants of the target agent(s) that the method can detect (Table 1).

Table 1. Ricin HHA: Inclusivity panel

No.	Variant
RC1	<i>Ricinus communis</i> agglutinin II (RCA 60 ^a) Antibody characterization panel ^b
RC2	<i>Ricinus communis</i> agglutinin II (RCA 120)
RC3	Ricin A chain
RC4	Ricin B chain
RC5	Ricin toxoid (vaccine surrogate)
RC6	Castor bean mash (cultivar 1)
RC7	Castor bean mash (cultivar 2)
RC8	Castor bean mash (cultivar 3)

^a RCA 60 is the actual ricin toxin.

^b The purpose of this panel is to characterize antibody activity. There are no criteria for detection.

Approved by AOAC SPADA on April 15, 2009.

Table 2. Ricin HHA: Exclusivity panel^a

No.	Identification
RCNN1	Abrin
RCNN2	Gelonin
RCNN3	Modeccin
RCNN4	Pokeweed protein
RCNN5	Saporin
RCNN6	Viscumin
RCNN7	Shiga toxin

^a Rationale: These are either structural or functional homologs of ricin, and thus potential cross-reactivities might be expected and need to be evaluated.

Approved by AOAC SPADA on January 23, 2009 with the provision that "wheat" flour is designated on the Environmental Factors Panel of Powders and Chemicals. July 21, 2009: *Speranskia*, *Chrozophora*, *Koilolepas*, and *Melanolepas* are not available.

2 Test Conditions

Test RCA 60 at AMDL. Test each member of the Antibody Characterization Panel at AMDL, except castor bean mash preparations, which are tested undiluted and at a 1/1000 dilution.

3 Acceptance Criteria

100% positive results.

Note: In the case of a negative result, retest 96 times with no failures allowed to demonstrate an estimated 5% lower confidence limit on the POD of 0.95 or higher. Data from testing the Antibody Characterization Panel is for informational purposes only.

Exclusivity

1 Definition

Nontarget agents, which are potentially cross-reactive, that are not detected by the method (Table 2).

2 Test Conditions

Test ricin exclusivity panel at 10 times AMDL.

3 Acceptance Criteria

100% negative results.

Note: In the case of a positive result, retest that panel member 96 times with no failures allowed to demonstrate a 95% upper confidence limit on the POD of 0.05 or lower.

Environmental Interference

1 Definition

Ability of the assay to detect RCA 60 in the presence of environmental substances and to be free of cross-reaction from environmental substances (*Annex A*).

2 Test Conditions

Test powders as liquid suspensions or solutions in the presence and absence of RCA 60 at the AMDL. Test swab materials in the presence and absence of RCA 60 at the AMDL.

3 Acceptance Criteria

No cross reactivity and no inhibition observed.

Note: In the case of a false-positive or false-negative result, retest the material 96 times with no failures.

Collaborative Validation Study

Reproducibility

1 Definition

Precision under conditions where independent test results are obtained with the same methods on equivalent test items in different laboratories with different operators using separate instruments.

2 Test Conditions

Test RCA 60 at AMDL and exclusivity panel member at 10 times AMDL. At least 12 replicates per material per collaborator with 12 collaborators (four collaborators at each of three test sites).

3 Acceptance Criteria

Must produce at least 10 valid data sets. Report standard deviation of reproducibility (s_R).

POD at the AMDL Under Reproducibility Conditions (formerly termed System False-Negative Rate)

1 Definition

Rate of positive system results in a population of known positive test portions.

2 Test Conditions

Test RCA 60 at AMDL. At least 12 replicates per collaborator with 12 collaborators (four collaborators at each of three test sites).

3 Acceptance Criteria

Data for target agent must demonstrate an estimated 5% lower confidence limit on the CPOD of 0.95 or higher, where CPOD is the probability of detection calculated from pooled valid collaborative data.

POD in the Absence of Analyte Under Reproducibility Conditions (formerly termed System False-Positive Rate)

1 Definition

Rate of positive system results in a population of known negative test portions.

2 Test Conditions

Test exclusivity panel member at 10 times AMDL. At least 12 replicates per collaborator with 12 collaborators (four collaborators at each of three test sites).

3 Acceptance Criteria

Data for near neighbor must demonstrate a 95% upper confidence limit on the CPOD of 0.05 or lower, where CPOD is the probability of detection calculated from pooled valid collaborative data.

Acknowledgments

All or part of this work was funded by the Department of Homeland Security Science and Technology Directorate, award HSHQDC-08-C-00012.

Ricin SMPRs (Version 4.2) approved by AOAC SPADA on August 3, 2009.

ANNEX A Environmental Factors Panel for Validating HHAs for Biothreat Agents

1 Powders and Chemicals

Bacillus thuringiensis powders (e.g., Dipel)
Powdered milk
Powdered infant formula (Fe fortified)
Powdered infant formula (low Fe formulation)
Powdered coffee creamer
Powdered sugar
Talcum powder
Wheat flour
Baking soda
Chalk dust
Brewer's yeast
Dry wall dust
Cornstarch
Baking powder
GABA (Gama aminobutyric acid)
L-Glutamic acid
Kaolin
Chitin
Chitosan
MgSO₄
Boric acid
Powdered toothpaste
Popcorn salt

2 Swab Materials

Cotton swab with plastic shaft
Rayon swab with plastic shaft
Macrofoam swab with plastic shaft
Method Developer sample collection device

Approved by AOAC SPADA on August 3, 2009.