NATO OTAN

NATO STANDARDIZATION AGENCY
AGENCE OTAN DE NORMALISATION

NSA

17 May 2004 NSA/0464-LAND/4571

STANAG 4571 LAND (EDITION 1) – SPECIFICATION FOR RAPID HAND-HELD ANTIBODY-BASED ASSAYS

Reference: AC/225-D/1462 dated 14 April 1999.

1. The enclosed NATO Standardization Agreement, which has been ratified by nations as reflected in the **NATO Standardization Document Database (NSDD)**, is promulgated herewith.

2. The reference listed above is to be destroyed in accordance with local document destruction procedures.

3. AAP-4 should be amended to reflect the latest status of the STANAG.

ACTION BY NATIONAL STAFFS

4. National staffs are requested to examine their ratification status of the STANAG and, if they have not already done so, advise Defence Investment Division, through their national delegation as appropriate of their intention regarding its ratification and implementation.

J. MAJ
Brigadier General, POLAR
Director, NSA

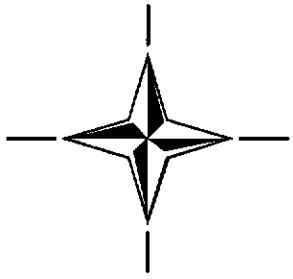
Enclosure:

STANAG 4571 (Edition 1)

NATO Standardization Agency – Agence OTAN de Normalisation
B-1110 Brussels, Belgium Internet site: http://nsa.nato.int
E-mail: landsection@hq.nato.int – Tel 32.2.707.4300

STANAG 4571
(Edition 1)

NORTH ATLANTIC TREATY ORGANIZATION
(NATO)



NATO STANDARDIZATION AGENCY
(NSA)

STANDARDIZATION AGREEMENT
(STANAG)

SUBJECT: SPECIFICATION FOR RAPID HAND-HELD ANTIBODY-BASED ASSAYS

Promulgated on 17 May 2004

J. MAJ
Brigadier General, POLAR
Director, NSA

RECORD OF AMENDMENTS

No.	Reference/date of Amendment	Date Entered	Signature

EXPLANATORY NOTES

AGREEMENT

1. This NATO Standardization Agreement (STANAG) is promulgated by the Director NATO Standardization Agency under the authority vested in him by the NATO Standardization Organisation Charter.

2. No departure may be made from the agreement without informing the tasking authority in the form of a reservation. Nations may propose changes at any time to the tasking authority where they will be processed in the same manner as the original agreement.

3. Ratifying nations have agreed that national orders, manuals and instructions implementing this STANAG will include a reference to the STANAG number for purposes of identification.

RATIFICATION, IMPLEMENTATION AND RESERVATIONS

4. Ratification, implementation and reservation details are available on request or through the NSA websites (internet http://nsa.nato.int; NATO Secure WAN http://nsa.hq.nato.int).

FEEDBACK

5. Any comments concerning this publication should be directed to NATO/NSA – Bvd Leopold III – 1110 Brussels – BE.

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NAVY/ARMY/AIR

NATO STANDARDIZATION AGREEMENT (STANAG)

SPECIFICATION FOR RAPID HAND-HELD ANTIBODY-BASES ASSAYS

Annexes: A. Reference Operations Requirements
B. List of Interferents, Simulants, and Cross-reactive Test Materials

Related Documents:

AC/224(LG/7)N/4 DATED 15 APRIL 1999 - PROCEDURES FOR PRODUCTION AND MANUFACTURE OF HAND-HELD ASSAYS

STANAG 2426 NBC - NUCLEAR, BIOLOGICAL AND CHEMICAL (NBC) HAZARD MANAGEMENT POLICY FOR NATO FORCES

AIM

1. The aim of this agreement is to specify the criteria for production and use of the Hand Held Antibody Based Test Kits (HHTKs).

AGREEMENT

2. Participating nations agree to adopt the characteristics of assays described in this agreement for the detection of important biological warfare agents in environmental and clinical samples. Assays produced under this agreement or in the future will adhere to these performance criteria as a minimum. This type of assay has not yet been approved for clinical use. Any country desiring approval for clinical use must process this assay through their individual medical testing and acceptance regime and provide the necessary resources to do so.

GENERAL DESCRIPTION OF HHTKs

3. Problem definition: Biotechnological advances afford NATO the capability to develop simple-to-use, robust, and fieldable HHTKs to permit the rapid monitoring and specific identification of toxins and infectious biological warfare agents at sensitivities comparable to standard ELISA format.

4. Characteristics of HHTKs: HHTKs are defined as : fast and simple to use antibody based hand held assays, providing unambiguous yes/no read-outs, not requiring trained operators either for sample application or for results/read-out interpretation. HHTKs will serve as a screening device, backed by more specific, precise, and possibly time consuming detection and identification methods. The HHTK is envisioned for use in environmental (and clinical laboratory applications) under usually occurring climatic and current meteorological conditions.

5. Operational requirements for development and evaluation were agreed upon by all Nations and are at Annex A.

TECHNICAL CHARACTERISTICS

6. General: This STANAG describes the current format of an immunochromatographic assay encompassing a membrane-linked antibody based sandwich assay utilizing capture antibody and detector antibody reagents : mass production of these assays may induce some changes in the format but not in the performance of the hand held test kits. The capture antibody reagent is printed as a line across a strip of nitrocellulose membrane. The membrane has a specific pore size and the ability to bind the capture antibody within its matrix. Liquid samples migrate through and on the nitrocellulose membrane strip at a specific rate based upon the pore size of the membrane. This assay system utilizes detector antibody coated on colloidal gold particles, size e.g. 40 nanometers. The detector antibody labeled colloidal gold particles are deep red in colour. They provide the reporter signal in the assay without the need for labile enzyme substrate reagents. Liquid sample is added to the sample well. This action places the solution in contact with the sample delivery pad. The liquid sample reconstitutes the lyophilized reagents and is delivered to the membrane. The solution wicks along the nitrocellulose membrane. If antigen is present in the sample, it will form a complex with the detector antibody-coated colloidal gold particle, producing a red line in the test window. This assay format incorporates a reaction control into the chromatographic assay strips by printing a line of antibody specific for the species of antibody coated on the colloidal gold particles. A positive assay will have two red lines, one at the site of capture antibody specific for antigen reaction and one at the site of the control line. A negative assay will have a single red line at the control site.

7. Performance requirements: See Annexes and B.

8. Stability: At least two years when properly packaged (Reference 1) and stored at 4°C.

9. Quality control and production: Assays produced in the USA will be in accordance with the manufacturer's existing internal quality assurance/quality control program, which has been accepted by the U.S. Army Joint Program Office for Biological Defense (JPO-BD). These functions will include but are not limited to: incoming inspection of purchased material, final inspection, maintenance of inspection and test records, and calibration records of all equipment used to inspect and test deliverables. A list of procedures for production of assay tests and quality control is included in Reference 1 only as a guideline. High volume production protocols and equipment will likely be somewhat different and will require approval by JPO-BD. The HHTKs produced in other countries must be in accordance with national procedures equivalent in scope and requirement to those described above for the USA. High volume production protocols and equipment will likely be somewhat different from those describe in Reference 1 and will require approval by JPO-BD, or equivalent body in other countries.

SAMPLE ACCEPTANCE

10. Sample acceptance testing for each production lot will be performed by JPO-BD (or equivalent for other countries) or a Government designated representative. JPO-BD (or equivalent for other countries) or representative will ship the assay kits to the NATO country buyer. Lot acceptance reports will be included with the assay kits.

PRICE

11. Price. The price per assay will be as stated in the applicable production contract, adjusted for cost of antibodies. This price will also include provisions for lot acceptance testing and reports, packaging, shipping, and administration. JPO-BD (or equivalent for other countries) reserves the right to adjust the price, depending on production quantity and manufacturing changes.

IMPLEMENTATION OF THE AGREEMENT

12. This STANAG shall be considered to be implemented when a nation has issued instructions that the criteria and procedures stated herein will be adopted for development, production and use of HHTKs.

ANNEX A TO
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REFERENCE OPERATIONAL REQUIREMENTS

1. The Hand Held Test Kits (HHTKs) are designed to detect high concentration of agents but cannot be used to declare an area free of contamination and safe for unprotected personnel.

2. Assay format and finalised assay must be compatible with varying environmental matrices listed below (solid samples and air must be presented to HHTK in a liquid form) :

a. Environmental matrices to be optimized

(1) Collected air
(2) Soil samples
(3) Surface water
(4) Hand surface samples
(5) Fabric samples
(6) Vegetation samples

b. Clinical and *postmortem* matrices: if utilized for clinical matrices the following may be applicable; human / animal samples i.e. swabs, body fluids (blood, nasopharyngeal, stool, etc.) and tissues

3. Assay format must require little or no sample pretreatment and concentrate on '1 step' formats.

4. Assay format must be capable of being performed in 15 minutes or less from time of sample application.

5. Sensitivity : Assay formats fulfilling above criteria will be evaluated based on sensitivity in 'stabilized' i.e. lyophilized end produce form. Sensitivity will be defined as the smallest amount of agent detected per ml of a sample with all the samples tested (i.e. 100% of repeat samples positive at assay sensitivity level). Sensitivity should be defined simultaneously with specificity.

6. Specificity : Assay formats in R&D and final production form must have greater than 95% specificity with negative samples. Less specificity will not result in a usable assay. High specificity should also be obtained with 'near neighbours' and unrelated antigens and 'interferents'.

7. Stability : Assay final formats must withstand 45%C for 6 weeks and maintain target sensitivity. Comparison in trials is only relevant for assay formats which meet this criteria through testing (will be agent assay specific to some degree).

8. Operating conditions : Assay formats must be compatible with the widest range of temperature and climatic conditions.

ANNEX B TO
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LIST OF INTERFERENTS, SIMULANTS AND CROSS-REACTIVE TEST MATERIALS (IN PBS, pH 7.2).

(ATCC# or other)

Bacteria :
1- *Bacillus anthracis*[(k)], Vollum (spores)
2- *Bacillus globigii* (spores)
3- *Bacillus megaterium* (spores) (# 14581)
4- *Bacillus subtilis* (spores)
5- *Bacillus thuringiensis*
6- *Brucella melitensis*[(k)] (#19396)
7- Burkholderia mallei[(k)]
8- *Coxiella burnetii*[(k)]
9- *Enterobacter agglomerans* (# 29904)
10- *Erwinia herbicola*[(k)]
11- *Escherichia coli* (# 11775)
12- *Francisella tularensis*[(k)]
13- *Pseudomonas aeruginosa*
14- *Staphylococcus aureus*
15- *Yersinia enterocolitica*
16- *Yersinia pestis*[(k)], F1 +
17- *Yersinia pestis*[(k)], F1 -
18- *Yersinia pseudotuberculosis*
19- *Vibrio cholerae*[(k)], Inaba strain

Toxin and Chemicals
20- Botulinum pentatoxoid
21- Bovine Serum Albumin
22- Malathion
23- Ovalbumin
24- Staphylococcal enterotoxin B
25-Tween 80 (0.1%)
26- Ricin

Viruses
27- MS2 bacteriophage
28- Vaccinia virus
29- Venezuelan equine encephalitis virus

Environmental
(in distilled water instead of buffer)
30- Air samples (smoggy)
31- *Aspergillus niger* (# 16404)
32- pollen, stage (# Sigma 9520)

Soils
33- sandy
34- loamy
35- clay

Smoke Particles
36- burning vegetation, wood
37- burning diesel fuel
38- burning fog oil
39- burning rubber
40- burning signal smokes

NB : [(k)] Agents killed by gamma irradiation.