World Health Organization

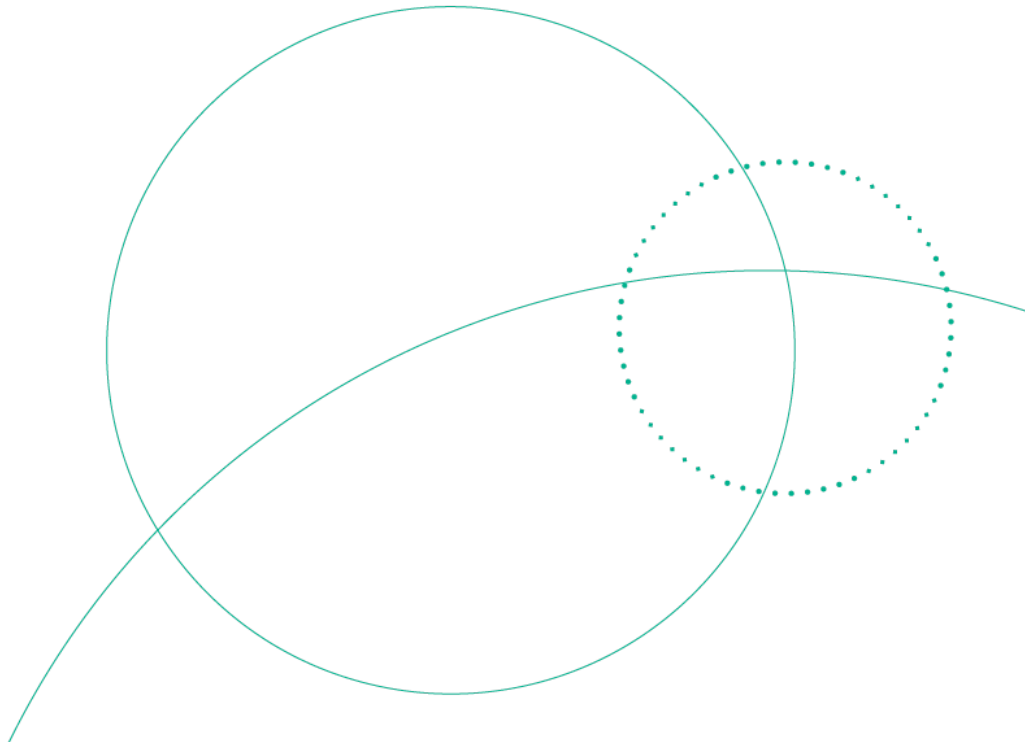
WHO Prequalification Programme / Vector Control Product Assessment

WHO Public Assessment Report: WHOPAR Part 3

GreenNet LN
(Shobikaa Impex Private Limited)

P-00320

Quality Assessment



Contents

1 Chemical and physical data

1.1 Chemical and physical properties

Data on the chemical and physical properties of the active ingredient and the product GreenNet LN were provided. These data were obtained from studies conducted according to established standards and/or Good Laboratory Practices (GLP) and are considered complete. Product specific properties are summarized in Table 1. Numerical results are presented as: mean (range). These summary results are based on the analysis of batches: GN01W1219, GN02W0120, GN03W0220, GN01W1021, GN02W1021.

Complete results from the study 21441 are available in Appendix 1.

Table 1. Chemical and physical properties for GreenNet LN (Batches: GN01W1219, GN02W0120, GN03W0220, GN01W1021, GN02W1021)

Data requirement	*Test method ID*	*Result*
Identification of deltamethrin	Deltamethrin CIPAC (N) 333/LN/(M)/2	The active ingredients each comply with an identity test
Deltamethrin mean content Accelerated storage deltamethrin retention	CIPAC (N) 333/LN/(M)/3	1.56 g/kg (1.50 -1.61 g/kg)* (1.47 - 1.63 g/kg)** 96.6% (95.7 -97.5%)*
Wash resistance index (Deltamethrin) Accelerated storage deltamethrin WRI	CIPAC (O) MT 195	95% (94.93 -95.07%)* 94.97% (94.92 -95.01%)*
Mesh size	See Appendix 2	26 (25-27) holes/cm^2 *
Fabric weight	ISO 3801/EN 12127 EN 20139-1992	42.1 g/m^2 (41.71- 42.48 g/m^2)*
Dimensional stability of netting to washing Accelerated storage dimensional stability	ISO3759-2011 / ISO5077-2007 / ISO6330-2012	Length: -1.9% (-2.2% to -1.6%)* Width: -2.4% (-2.6% to -2.1%)* Length: -2% (-2.3% to -1.7%)* Width: -2.5% (-2.6% to -2.3%)*
Bursting strength Accelerated storage bursting strength	ISO 13938-2-1999 (30mm Diaphragm Diameter)	426.8 kPa (403.0 - 450.6 kPa)* 424.2 kPa (400.3 - 448.0 kPa)*
Seam bursting strength Accelerated storage seam bursting strength	ISO 13938-2-1999 (30mm Diaphragm Diameter)	459.1 kPa (417.0 - 501.2 kPa)* 458.5 kPa (412.8 - 504.2 kPa)*
Flammability	EN 1102:2016	No ignition or propagation. Maximum hole width 25 mm, length 50 mm.

* range of means
** range of individual measurements in samples

No significant differences were recorded among the properties of the product kept at ambient temperature and after accelerated storage stability test conditions.

1.2 Manufacturing, composition and formulant information

Data on the manufacturing process and product composition for GreenNet LN have been provided and are adequate. A summary is presented in Table 2. Detailed information on the manufacturing process and product formulation is considered Confidential Business Information (CBI).

Table 2. Manufacturing process and product composition data submitted for GreenNet LN

Description of starting material	Deltamethrin SC. The source of active ingredient is supported by a current evaluation report confirming compliance of the material with the established WHO specification. Polyester multifilament yarn produced from recycled PET bottles.
Declaration of product formulation	Included in the confidential business information.
Production / formulation process	The fabric is manufactured by warp-knitting of the polyethylene terephthalate (PET) (common name polyester) yarn, heat setting, mixing of the AI SC with the binder formulation, and coating. The finished product is manufactured by cutting and sewing of the fabric, addition of label tag, folding prior to packaging, packing, and baling.
Packaging	Nets may be packed individually in 38 cm × 46 cm polyethylene bags with printed labels or tied into bundles of five loose nets prior to baling with one adhesive label per bale (40/50 nets may be baled together, tightly bound with PET strap). The sizes of the bags and bales may vary depending on the customer requirements.
Discussion of impurities	There are no relevant impurities of toxicological concern.
Certification of limits	Deltamethrin: 1.4 g/kg, acceptable limits 1.05-1.75 g/kg

1.3 Enforcement analytical method

Table 3. Details of the analytical method used to determine Deltamethrin in GreenNet LN

Quantification of deltamethrin	Deltamethrin: CIPAC (N) 333/LN/(M)/3

These methods are appropriate for the determination of the active ingredient content of the product.

2 Chemical and entomological fabric characterisation

Laboratory studies to characterize the availability of the active ingredient and the insecticidal effect of the fabric of GreenNet LN on Anopheline mosquito species were submitted to WHO as part of the prequalification dossier. Sampled pieces of ITNs used in the biological laboratory studies were characterized for their deltamethrin content using HPLC.

2.1 Laboratory studies

2.1.1 Entomological characterisation

Data on the wash regeneration and wash resistance properties of the GreenNet LN product were provided. These data were obtained from studies conducted according to established standards and/or Good Laboratory Practices (GLP). These summary results are based on ITNs drawn from batches GN01W1219 and GN02W0120.

One laboratory study was submitted to characterise the GreenNet LN fabric. Bioavailability was evaluated in wash regeneration and wash resistance studies. The endpoint used to evaluate bioavailability was 24-hour mortality. Demonstration of bioavailability post-wash was used to determine the wash interval that was selected for use in the wash resistance study. Thresholds of ≥95% knockdown and/or ≥80% mortality in WHO cone tests were used to determine wash resistance.

The bioavailability of deltamethrin on the surface of the ITN was characterized using the insecticide susceptible test system *Anopheles stephensi*. WHO cone tests was the experimental method used in bioavailability studies.

The wash interval was determined to be one day. Using this wash interval, GreenNet LN was wash resistant to 20 washes using an insecticide susceptible mosquito test system.

These results are summarized in Tables 4 and 5.

Table 4. Wash regeneration study results for GreeNet LN using the insecticide susceptible *An. stephensi* test system in WHO cone tests to characterize the bioavailability of deltamethrin.			
Days post-wash	*An. stephensi*		
	n	%KD (95% CI)	%M24 (95% CI)
UW	200	100	100
0	200	100	100
1	200	100	100
2	200	100	100
3	200	100	100
5	200	100	100
7	200	100	100

Table 5. Wash resistance study results for GreenNet LN using insecticide susceptible *An. stephensi* mosquitoes using WHO cone tests			
Wash No.	*An. stephensi*		
	n	%KD (SE)	%M24 (SE)
0	200	100	100
1	200	100	100
3	200	100	100
5	200	100	100
10	200	98 (0.00)	93.5 (0.96)
15	200	92 (0.82)	88 (0.82)
20	200	87.5 (0.96)	84 (1.41)

Chemical characterization of samples

Data on the deltamethrin content of sampled pieces of the GreenNet LN product used in the entomological laboratory wash resistance study and the supplemental laboratory study were provided. These data were obtained from studies conducted according to established standards and/or Good Laboratory Practices (GLP). These summary results are based on ITNs drawn from batches GN01W1219 and GN02W0120. The results are summarized in Table 6.

Table 6. AI content and retention of sampled pieces of GreenNet LN used in the entomological wash resistance study (batch numbers GN01W1219 and GN02W0120)

Wash No.	Mean deltamethrin content (g/kg)	RSD (%)	Deltamethrin retention	Deltamethrin retention per wash
0	1.49 (1.47-1.51)	1.90	-	-
1	1.47 (1.44-1.50)	2.89	98.7	98.7
3	1.41 (1.38-1.43)	2.52	94.6	98.2
5	1.31 (1.27-1.34)	3.79	87.9	97.5
10	1.12 (1.09-1.15)	3.79	75.2	97.2
15	0.99 (0.97-1.00)	2.15	66.4	97.3
20	0.92 (0.91-0.92)	0.77	61.4	97.6
25	0.82 (0.81-0.83)	1.72	55.0	97.6

The mean AI content presented in Table 6 was determined based on 5 pieces per net x 2 nets per batch x 2 batches, indicating ranges to the AI content in parenthesis.

AI retention per wash in Table 6 is calculated as:

- AI retention per wash = 100 x $^{n}\sqrt{(t_n/t_0)}$ where:
  - t_n = total active ingredient content after n washing cycles
  - t_0 = total active ingredient content before washing
  - n = number of washes.

2.2 Chemical and entomological fabric characterisation conclusions

The submitted laboratory studies characterize the fabric of GreenNet LN against an insecticide susceptible strain of *An. stephensi* mosquitoes. Following three washes intended to deplete the surface of the fabric of bioavailable insecticide, the laboratory results demonstrate that sufficient bioavailable insecticide to induce mortality in the insecticide susceptible test system was present one day after washing.

Wash resistance to 20 washes using standardised washing methods was demonstrated against an insecticide susceptible strain of *An. stephensi* mosquitoes. Based on the submitted studies, GreenNet LN is wash resistant to 20 washes using a one-day wash interval against insecticide susceptible *An. stephensi.*

3 Overall quality conclusions

Based on the studies and information provided, all data requirements for the prequalification assessment of product quality have been satisfied. These data have been relied upon to assess the formulation, manufacturing process, and physical/chemical characteristics of the proposed product for the purpose of establishing the identity of the product and assuring that the product can be produced consistently.

The methods for assessing the physical/chemical properties of the product were CIPAC methods and/or validated methods.

The quality component of the dossier is considered complete, and the assessment of the submitted information on quality supports prequalification of the product.

Table 7. List of studies submitted to WHO as part of the prequalification dossier

Studies that were relied upon for decision making	
Study number	Study title
20128	GreenNet - Deltamethrin 1.4 g/kg coated onto polyester filament of Long-Lasting Insecticidal Net: Regeneration, Wash Resistance and Efficacy against Mosquito, *Anopheles stephensi*
20124	GreenNet - Deltamethrin 1.4 g/kg coated onto polyester filament of Long-Lasting Insecticidal Net: Analysis of Deltamethrin in the Net pieces following the Bio-efficacy Phase I study
21441	GreenNet - Deltamethrin 1.4 g/kg coated onto polyester filaments of Long Lasting Insecticidal Net: Analytical Test Report
Studies that were not used to inform decision making	
20125	GreenNet - Deltamethrin 1.4 g/kg coated onto polyester filaments of Long Lasting Insecticidal Net: Analytical Test Report (this study was superseded by study 21441)

4 Manufacturing release specifications

4.1 Summary of manufacturing release specifications

Table 8. Summary of manufacturing release specifications

Description
The material shall be in the form of netting*, consisting of 100 denier* multi-filament polyester yarn, treated with technical deltamethrin complying with the requirements of WHO specification 333/TC (current version) together with any necessary other formulants. The product shall appear clean and shall be free from visible extraneous matter,* visible damage (such as splitting or tearing) and visible manufacturing defects (such as poorly made seams or a weave that is either not uniform or too loose to remain uniform in use) and shall be suitable for use as an insecticidal net with long-lasting activity*.

ID	*Property*	*Method*	*Declared value*
1*	Sampling Plan	See Appendix 2	
2*	Deltamethrin content	CIPAC (N) 333/LN/(M)/3, p. 66 2009	1.4 g/kg ± 25%
3*	Deltamethrin wash resistance index	MT195, CIPAC Handbook O, p. 205, 2017	Within the range 80% to 98%
4	Fabric weight	ISO 3801 / EN 12127	40 g/m^2 ± 10%
5*	Bursting strength – fabric	ISO 13938:2	Not less than 350 kPa
6*	Bursting strength – seam	ISO 13938:2	Seam bursting strength average shall be not less than the average bursting strength for fabric
7*	Netting mesh size	See Appendix 2	Average ≥ 24 holes/cm^2 Min. 24 holes/cm^2
8*	Dimensional stability of netting to washing	See Appendix 2	Not more than 10% shrinkage and not more than 5% expansion in both directions.

* Indicates that additional information is available in Appendix 2.

Manufacturers are expected to rely on the information above as part of a QC management plan and for validation of product quality when released. To the extent required, Certificates of Analysis to support the release of products should present results for the attributes identified in the above table.

4.2 Storage

Accelerated storage stability data were generated as per CIPAC MT 46.3. Test samples were stored for 14 days at 54°C. No significant differences were recorded among the properties of the product kept at ambient temperature and after accelerated storage stability test conditions.

Products should be stored and transported in appropriate conditions in accordance with the recommendations of the manufacturer.

Where products have been subjected to prolonged storage or adverse conditions during storage, analysis and testing are recommended to assess changes in characteristics and their suitability for use.

Appendix 1. Summary of available data considered in Module 3

Batches used to generate the physical/chemical data

Batch Number	Date	Formulation	Uses
GN01W1219	06/12/2019	White	Storage stability, in-use stability (laboratory)
GN02W0120	22/01/2020	White	Storage stability, in-use stability (laboratory)
GN03W0220	25/02/2020	White	Storage stability
GN01W1021	10/2021	White	Storage stability
GN02W1021	10/2021	White	Storage stability

Product characteristics

Study 21441:

Property	Batch ID	Test Method	Results
Deltamethrin mean content	GN01W1219	CIPAC 333/LN/(M)/3	1.52 g/kg (RSD 1.99%)
	GN02W0120		1.59 g/kg (RSD 1.07%)
	GN03W0220		1.50 g/kg (RSD 0.96%)
	GN01W1021		1.57 g/kg (RSD 1.04%)
	GN02W1021		1.61 g/kg (RSD 0.98%)
Deltamethrin distribution (Numbers in parenthesis indicate percentage of target dose)	GN01W1219		1.47-1.55 g/kg (105.0-110.7%)
	GN02W0120		1.57-1.61 g/kg (112.1-115.0%)
	GN03W0220		1.48-1.52 g/kg (105.7-108.6%)
	GN01W1021		1.55-1.59 g/kg (110.7-113.6%)
	GN02W1021		1.59-1.63 g/kg (113.6-116.4%)
Wash resistance index (WRI)	GN01W1219	CIPAC MT195	94.95%
	GN02W0120		95.04%
	GN03W0220		95.01%
	GN01W1021		95.07%
	GN02W1021		94.93%
Fabric weight	GN01W1219	ISO 3801	42.48 g/m2
	GN02W0120		41.91 g/m2
	GN03W0220		42.02 g/m2
	GN01W1021		41.71 g/m2
	GN02W1021		42.21 g/m2
Mesh size	GN01W1219	ISO 139	25-26 holes/cm2
	GN02W0120		25-26 holes/cm2
	GN03W0220		25-26 holes/cm2
	GN01W1021		26-28 holes/cm2
	GN02W1021		26-27 holes/cm2
Dimensional stability	GN01W1219	ISO 3759/5077/6330	-1.7%, -2.1%
	GN02W0120		-1.7%, -2.2%
	GN03W0220		-1.6%, -2.3%
	GN01W1021		-2.2%, -2.6%
	GN02W1021		-1.8%, -2.2%
Bursting strength	GN01W1219	ISO 13938-2	420.1 kPa
	GN02W0120		413.4 kPa
	GN03W0220		403.0 kPa
	GN01W1021		450.6 kPa
	GN02W1021		432.9 kPa
Seam bursting strength	GN01W1219	ISO 13938-2	445.2/423.2 kPa
	GN02W0120		439.8/421.9 kPa
	GN03W0220		430.2/417.0 kPa
	GN01W1021		493.2/480.7 kPa
	GN02W1021		501.2/490.3 kPa

Property	Batch ID	Test Method	Results
Flammability	GN01W1219 GN02W0120 GN03W0220 GN01W1021 GN02W1021	EN 1102:2016	No ignition or propagation. Maximum hole width 25 mm, length 50 mm.

Storage stability

Study 21441:

Property	Batch ID	Before	After	Change
Deltamethrin content	GN01W1219	1.52 g/kg	1.47 g/kg	-0.05 g/kg (-3.3%)
	GN02W0120	1.59 g/kg	1.55 g/kg	-0.04 g/kg (-2.5%)
	GN03W0220	1.50 g/kg	1.46 g/kg	-0.04 g/kg (-2.7%)
	GN01W1021	1.57 g/kg	1.51 g/kg	-0.06 g/kg (-3.8%)
	GN02W1021	1.61 g/kg	1.54 g/kg	-0.07 g/kg (-4.3%)
Wash resistance index	GN01W1219	94.95%	94.96%	+0.01%
	GN02W0120	95.04%	94.92%	-0.13%
	GN03W0220	95.01%	94.97%	-0.04%
	GN01W1021	95.07%	94.94%	-0.13%
	GN02W1021	94.93%	95.01%	+0.08%
Dimensional stability	GN01W1219	-1.7%, -2.1%	-2.0%, -2.3%	-
	GN02W0120	-1.7%, -2.2%	-1.7%, -2.4%	-
	GN03W0220	-1.6%, -2.3%	-1.9%, -2.5%	-
	GN01W1021	-2.2%, -2.6%	-2.3%, -2.6%	-
	GN02W1021	-1.8%, -2.2%	-1.8%, -2.3%	-
Bursting strength	GN01W1219	420.1 kPa	428.2 kPa	+8.1 kPa
	GN02W0120	413.4 kPa	412.5 kPa	-0.9 kPa
	GN03W0220	403.0 kPa	400.3 kPa	-2.7 kPa
	GN01W1021	450.6 kPa	448.0 kPa	-2.6 kPa
	GN02W1021	432.9 kPa	438.8 kPa	+5.9 kPa
Seam bursting strength	GN01W1219	≥ 423.2 kPa	≥ 432.2 kPa	+9.0 kPa
	GN02W0120	≥ 421.9 kPa	≥ 417.8 kPa	-4.1 kPa
	GN03W0220	≥ 417.0 kPa	≥ 412.8 kPa	-4.2 kPa
	GN01W1021	≥ 480.7 kPa	≥ 485.6 kPa	+4.9 kPa
	GN02W1021	≥ 490.3 kPa	≥ 492.2 kPa	+1.9 kPa

Appendix 2. Manufacturing release specifications: methods and notes

Description

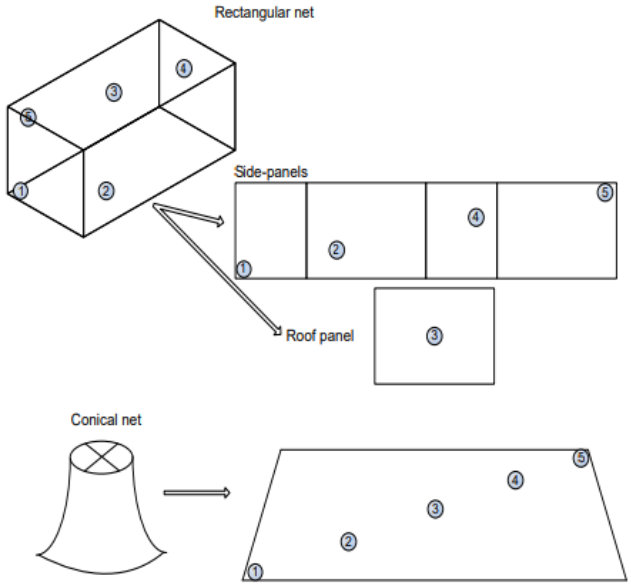
- The specification applies to netting in bulk and manufactured nets. The netting may be white or coloured, for example, blue or green.
- The linear density (denier) of the yarn cannot be measured in the netting or the manufactured bed net but it should be identified on the packaging.
- Occasional short lengths of loose thread present in the netting are not considered to be extraneous matter.

Sampling Plan – Applicable to all attributes for which samples are to be taken from various parts of the constructed ITN.

- Sampling should be noted as Figure 1:

Figure 1 General method for sampling rectangular and conical nets

Recommended positions from which 5 pieces of netting should be taken from a made up bed net and combined to form a representative sample.



Samples should be taken according to Figure 1 or on a convenient diagonal across the width of bulk material. Samples must be sufficiently large to conduct all tests required and representative of the net or netting. Except where seams are to be tested, do not test material within 10 cm of seams or selvedges.

Use sharp scissors, or equivalent, to minimize damage to the fibres and fabric and thus avoid any consequential bias in the results of certain tests. Roll up the strips or squares and place them in labelled,

new, clean aluminium foil prior to analysis. Samples should be kept cool, avoiding heat sources (including direct sunlight) or freezing, and analyzed/tested with minimum delay. Representative portions (sub-samples) for testing should be taken as described in each test method.

Attribute 2: Deltamethrin content

For complete identification and good quantification, deltamethrin which is a single pyrethroid stereoisomer consisting of [αS,1R,3R]-isomer (also known as the S-isomer) must be separated from the [αR,1R,3R]-isomer (otherwise known as the R-isomer), which is not part of the active ingredient and not a relevant impurity. These diastereomers may be separated by non-chiral techniques as provided in the CIPAC method for deltamethrin.

The deltamethrin content may be declared as both g/kg and mg/m2 but, in case of dispute, g/kg values shall be used. If the active ingredient content is also specified as mg/m2 of netting material, the actual content on this basis is calculated from the measured values for active ingredient content in g/kg and mass of net/m2. Mass of net/m2 should be determined according to ISO 3801 / EN 12127.

Attributes 3: Deltamethrin wash resistance index

The content of deltamethrin in the net pieces before and after washing should be determined by the method 333/LN/(M)/3, CIPAC Handbook M, p.66, 2009.

Attributes 5 and 6: Bursting strength – fabric and bursting strength – seam

Test method: ISO 13938 part 2 with conditioning of the fabric as specified in the ISO standard. The declared bursting strength, and testing for compliance with it, should be based on tests of 7.3 cm^2 areas of fabric. Proposed specifications based on tests of 50 cm^2 area must be supported by data showing the suitability of the proposed value and its relationship to minimum of 250 kPa (which is based on 7.3 cm^2 area). Five replicate tests should be conducted on samples taken at approximately equal distances on a diagonal across the netting, taking no sample within 10 cm of a border or seam. In made up rectangular nets, the “diagonal” may correspond to figure 1. The average of the 5 measurements is calculated.

The method to test seam bursting strength is identical to that used to test the fabric, except that 5 replicate tests should be made, with the seam centred on the test head. Up to 5 seams may be tested but, if there are < 5 seams, replicate measurements should be made on 1 or more seams, to provide a total of 5 measurements.

Attribute 7: Mesh size

In the absence of a simple or standard method to determine the size of holes, which may have complex shapes, in highly flexible fabrics, mesh size is determined by counting the number of holes in a square of the fabric. Counting may be done directly on the fabric or indirectly by taking a picture/photocopy of the fabric. Indirect methods may ease counting and provide a permanent record. The number of holes per measured area is converted in holes/cm2. Before counting, the fabric should be conditioned according to ISO 139 (4 h, 20ºC, 65% relative humidity).

Use a template to define the square of netting, taking care not to stretch or distort the fabric. The template should be a 1-2 mm thick rigid sheet, in/on which an accurately calibrated (±1% in each

dimension) square (e.g., 1 x 1 in or 5 x 5 cm) has been cut/marked. If a template is not available and a ruler must be used, great care is required to ensure that the area counted is square. Where practicable, one edge of the square to be counted should be aligned with a row of complete holes in the fabric. Incomplete holes ≥ ½ are counted as complete holes, whereas those < ½ are not counted. Count 5 replicate squares selected according to the sampling plan, calculate the average and note the lowest value.

Another suitable method is the use of a stereomicroscope with an image analyser software, where the number of holes in a defined area is counted. In case of discrepancy between the netting mesh size using stereomicroscopic method and direct or indirect counting method, the stereomicroscopic method shall be the referee method.

Attribute 8: Dimensional stability of netting to washing

Method of preparation, marking and measuring: ISO 3759. Method of washing: ISO 6330. Method of calculation: ISO 5077. Size of test portions: 500 mm x 500 mm; mark off 350 mm x 350 mm within each test portion. Test a total of 4 replicate portions, 2 washed in each of 2 separate loads. Type of washing machine: ISO type A (front loading). Washing programme: 30ºC Mild programme. Fill the washer with fabrics and ballast Type III (polyester ballast) up to 2 kg (according to the ISO 6330 standard). Drying: flat drying.